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BRIEF COMMUNICATIONS

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Identification and Characterization of a Novel Polypeptide in the CNS of *Bombyx mori* During Pupal - Adult Transformation

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Abstract: Detailed one and two dimensional electrophoresis (PAGE) studies carried out on the CNS proteins of *Bombyx* revealed the appearance of a new nervous system specific acidic polypeptide with a molecular mass of 235 kDa in the pharate adults. This polypeptide is totally absent in the late larval, early pupal and mid pupal stages. Synthesis of this polypeptide begins in pharate adults and its concentration increases gradually during the adult development and it reaches a very high concentration in 48 h old moths.

Keywords: Metamorphosis; cell death; nervous system protein; *Bombyx mori*

INTRODUCTION

Naturally occurring cell death or programmed cell death is an important feature of developing and metamorphosing nervous system of insects which regulates the size of neuronal population (Truman, 1984; Booker and Truman, 1987; Fahrbach and Truman, 1987). Cell death is required to eliminate the cells which are no longer required as well as for the generation of new cells having specific characteristics. The steroid hormone 20-hydroxyecdysone (20-HE) plays an important role in controlling post-metamorphic neuronal death in *Manduca sexta* (Truman and Schwartz, 1984). Furthermore, *in vivo* and *in vitro* studies using RNA and protein synthesis inhibitors reveal that *de novo* synthesis is required for the post-embryonic neuronal death (Fahrbach and Truman, 1987). The same has been found to be true for the programmed cell death in the inter segmental muscles in *Manduca* (Schwartz *et al.*, 1990).

There is little doubt that much progress has been made towards understanding the process of programmed cell death in insects and significantly towards the actual role of metamorphic hormone (20-HE) in this process. Several approaches are currently being pursued. These include attempts to find mRNAs and/or proteins whose increased expression / synthesis is associated with cell death. Recently, a 40 kDa protein has been identified and implicated with neuronal death in *Manduca* (Montemayor *et al.*,

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1990). In the present study an attempt has been made to analyse the pattern of CNS proteins in *Bombyx mori* using one and two dimensional polyacrylamide gel electrophoresis(PAGE) and silver staining during pupal-adult transformation. At this time, the CNS contains a mixture of neurons that will survive during adult life, neurons that are already degenerating, and neurons that are committed to die but have not yet begun the process of degeneration (Truman, 1983; Truman and Schwartz, 1984). Thus, any changes in protein pattern detected at this time could reflect changes in protein expression in any of these three cell populations.

MATERIALS AND METHODS

Insects:

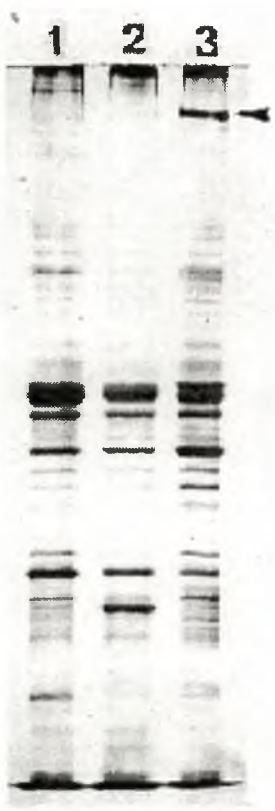
Third instar larvae of *Bombyx mori* (pure Mysore strain) were obtained from local breeding centre and reared in an insect culture room at 26 ± 1°C temperature, 70 ± 5% relative humidity and 14 h: 10 h light-dark period, on fresh mulberry leaves. For the present study, late-last instar larva, pre-pupa, mid-pupa, pharate adult (late-pupa) and adult stages were used.

Preparation of tissue sample:

The intact nervous system and other tissues - muscle, alimentary canal and salivary glands were rapidly dissected out and homogenized in chilled homogenization buffer (10mM Tris; 0.1% Triton X-100; pH 7.1) using a glass microhomogenizer (Kontes). The homogenate was centrifuged at 1000 x g for 2-3 min in a microfuge at 4°C. The supernatant was collected and used for protein estimation and one dimensional electrophoresis. Before electrophoresis, sample was mixed with an equal amount of 2x sample buffer (containing 0.125 M Tris-HCl, pH 6.8; 4% SDS; 20% glycerol; 10% 2-mercaptoethanol and 0.002% bromophenol blue) and incubated at 100°C for 1 min. For two-dimensional electrophoresis, the tissue was homogenized in IEF sample buffer containing 9.5 M urea, 2% LKB carrier ampholytes (comprised of 1.6% 5/7 pH and 0.4% 3.5/10 pH), 2% NP-40 and 5% 2-mercaptoethanol.

Electrophoresis:

One dimensional SDS-PAGE was carried out according to the procedure of Laemmli (1970). A 1cm 3.3% stacking gel (pH 6.8) was followed by a 15 cm separating gel (pH 8.8). Tris-glycine buffer (0.025 M) with 0.1% SDS (pH 8.3) was used as the electrode buffer. Proteins were visualized by silver staining (Blum *et al.*, 1987). Two dimensional gel electrophoresis was performed as described by O'Farrell (1975). Ampholyte polyacrylamide tubes were prefocused for 1 h at 200 V to set-up the pH gradient. The gels were run for a total of 10,000 V h. The anolyte used was 0.01 M H_3PO_4, the catholyte used was 0.02 M NaOH. The gels were subsequently transferred to 5 ml equilibration buffer containing 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS and 0.0625 M Tris-HCl (pH 6.8) and stored at -20°C. The second dimension separation was carried out using 3.3% stacking gel and 10% separating gel. High molecular weight (Sigma) standards were run at the acidic end of several gels. The gels were run at 25mA/gel until the bromophenol blue dye reach the bottom of the gel. The gels were fixed and silver stained. To determine the pH gradient of IEF gels, parallel gels were cut into pieces of 0.5 cm length and incubated for 2 h in 0.5 ml degassed



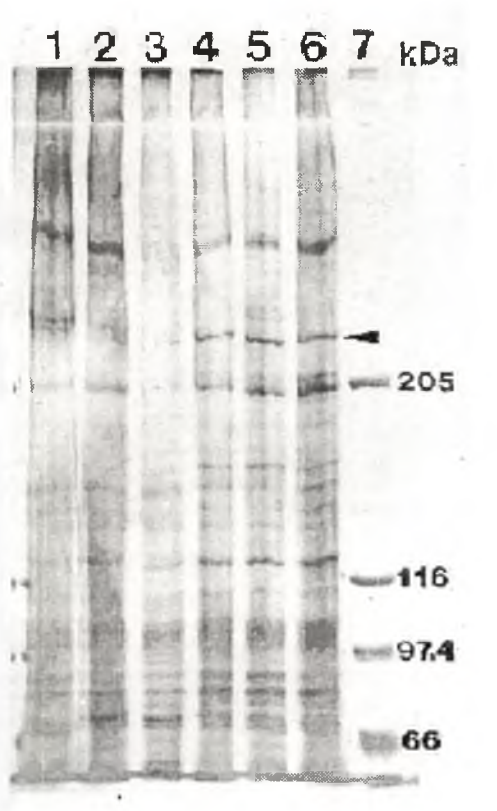


Fig.1 Fig. 2

Fig. 1 shows the polypeptide pattern from the CNS of late-last instar larvae (lane 1), mid-pupa (lane 2) and adult (lane 3). The protein was separated on a 10% SDS-PAGE, and in each lane 10 μg protein was loaded. Note the presence of a 235 kDa (◀)polypeptide in the CNS of adult moths. Fig. 2 shows the polypeptide pattern in the CNS during various stages of development. 10μg protein sample from each stage was separated on a 5% SDS-PAGE. The sample loaded in lane 1 is from pre pupa, lane 2 from early pupa, lane 3 from pharate adult (late-pupa), lane 4 from 12 h old adult, lane 5 from 24 h old adult, and lane 6 from 48 h old adult. Note that the concentration of the 235 kDa polypeptide (◀)increases in the CNS during the pupal and adult development. Lane 7 shows high molecular weight markers (Sigma).

distilled water. The pH was measured electrometrically. Densitometric scanning of one dimension gels were done with a Bio-Med soft laser scanning densitometer. For all studies equal quantity of protein samples were loaded and comparisons were made. Protein was estimated according to the method of Bradford (1976) in the case of samples for one dimensional electrophoresis and according to the method of Ramagli and Rodriguez (1985) for two dimensional electrophoresis, which allows the quantitation of the proteins in the presence of urea and carrier ampholytes.

RESULTS

Detailed electrophoretic (SDS-PAGE) studies carried out on the CNS proteins of *Bombyx* during pupal-adult transformation revealed the appearance of a new nervous system specific polypeptide with an apparent molecular weight of 235 kDa in pharate

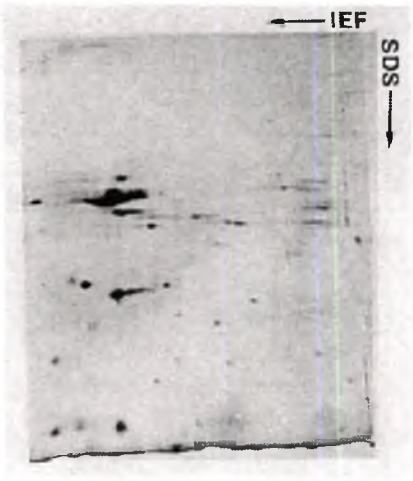


Fig. 3

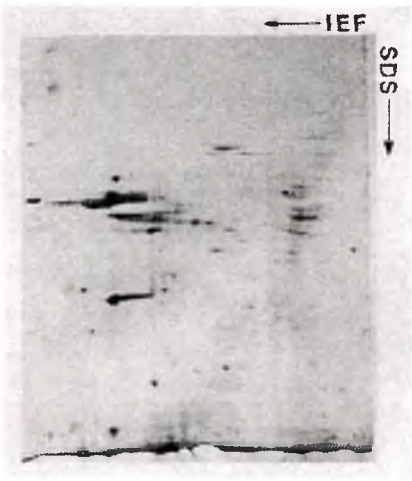


Fig. 4

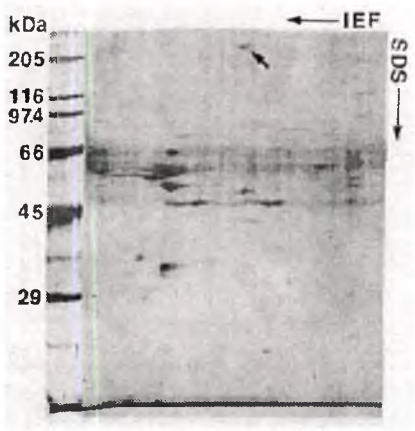


Fig. 5

Figs. 3, 4 and 5 – Photographs showing the fractionation of CNS proteins from late-last instar larva (Fig. 3), early-pupa (Fig. 4) and adult (Fig. 5) on two dimensional polyacrylamide gel. The proteins were separated between 20 to 260 kDa on vertical axis and 4.7 to 7.2 p*I* on horizontal axis. Note the presence of a new protein (⇐) which appears in the CNS of adult insect.

adults. This polypeptide was totally absent in the late-last larval, prepupal and early and mid-pupal stages (Fig. 1, lanes 1 & 2; Fig. 2, lanes 1 & 2). Laser scanning densitometry of dried gels indicated that this polypeptide was present in low concentration in pharate adult (Fig. 2, lane 3) and its content gradually increases during adult development and reaches highest in 48 h old moths. The concentration of this protein remains more or less the same up to 4-5 days (which is the total life span of the adult moth). All the experiments were repeated thrice with tissue samples from three different batches. Subsequent analysis of CNS proteins by two dimensional electrophoresis clearly showed that this 235 kDa polypeptide was expressed only during the pharate

adult and adult development (Fig. 5) and it is totally absent during the larval (Fig. 3) and early pupal (Fig. 4) development. The p*I* value of the polypeptide ranges between 6-6.2. Furthermore, this polypeptide is found to be absent in other tissues like muscles, salivary glands and alimentary canal (data not presented) of larval, pupal and pharate adult stages.

DISCUSSION

Programmed cell death has been observed during the development of virtually all metazoan organisms. Extensive studies in *C. elegans* suggest that highly regulated genetic programme is responsible for programmed cell death and the proteins encoded by ced-3 and ced-4 genes act within the dying cells themselves and/or interact with other intracellular molecules to produce cell death (Ellis *et al.*, 1991). While another gene ced-9 product, acts antagonistically to ced-3 and ced-4 products to suppress the cell death (Fanidi and Evan, 1994). DNA fragmentation has been demonstrated in some models of excitotoxin and neurotoxin induced cell death (Dispasquale *et al.*, 1991; Kure *et al.*, 1991) as well as programmed neuronal death (Clarke and Hornung, 1989; Johnson *et al.*, 1989). Furthermore there increasing evidence for the possible role of nucleases in DNA fragmentation during neuronal death (Ashwell *et al.*, 1994).

In this study we have identified a novel 235 kDa acidic polypeptide in the CNS of *Bombyx mori*, which shows a precise developmental regulation. The synthesis of the polypeptide begins at pharate adult stage and it may play a role in programmed cell death. Montemayor *et al.*, (1990) have reported the appearance of a 40 kDa acidic protein in *Manduca* during neuronal death. However, the apparent molecular weight of the newly synthesized polypeptide in *Bombyx* seems to be nearly six times greater than that reported for *Manduca*. Programmed cell death in the intersegmental muscles of *Manduca* is shown to be suppressed by inhibitors of macromolecular synthesis indicating that it requires new RNA and protein synthesis (Lockshin, 1969; Schwartz *et al.*, 1990).

In conclusion, in the present study we have demonstrated the expression of a developmentally regulated new tissue specific polypeptide which may play a role in CNS remodelling during metamorphosis of *Bombyx mori*. Further studies on identification of protein and its cellular distribution should provide an insight into whether its expression is actually related to the commencement of neuronal death.

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Scanning Electron Microscopic Studies of the Stridulatory Apparatus of the Coconut Rhinoceros Beetle *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae)

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Abstract: Ultramorphometric characteristics of the elytro-abdominal stridulatory apparatus of the adult male and female *Oryctes rhinoceros* are being reported. The elements of the abdominal stridulatory structure (the *plectrum*) revealed marked degree of sexual dimorphism. However the components of the elytral stridulatory structure (the *pars stridens*) did not disclose any noticeable difference between the sexes. These aspects are discussed in detail, in the light of the data available on other coleopterans.

Keywords: *Oryctes rhinoceros*; Stridulatory apparatus; *plectrum; pars stridens*; Scanning Electron Microscopic Studies.

INTRODUCTION

Oryctes rhinoceros L. is a highly menacing pest of the coconut palm *Cocos nucifera* L. During a study made by the present author on the reproductive behaviour of this beetle it was found that male stridulatory behaviour constituted an invariable component of courtship and mating behaviour in this insect and subsequent studies revealed several aspects of its stridulatory behaviour such as the occurrence of different kinds of stridulations produced by both sexes under different contexts, general mechanism of sound production as well as morphology of the stridulatory apparatus as disclosed by light microscopy (Mini and Prabhu, 1990).

In the elytro-abdominal type of stridulation, the abdominal component of the stridulatory apparatus is usually referred to as the *plectrum* while the elytral component is known as the *pars stridens* (Gibson, 1967; Harman and Harman, 1972; Michael and Rudinsky, 1972; Selander and Jansson, 1977). Sexual dimorphism of the stridulatory structures are very often indicative of sexual differences in the physical attributes of stridulatory signals, as observed in coleopterans like *Conotrachelus* (Gibson, 1967)

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and *Lema trilineatadaturaphila* (Kogan *et al.* 1970). Sex-dependent difference regarding the kinds and contexts of stridulation has already been reported in *O. rhinoceros* (Mini and Prabhu, 1990)

The present study elucidates the ultramorphological details of the stridulatory apparatus of *O. rhinoceros* beetles giving special emphasis on aspects of its sexual dimorphism.

MATERIALS AND METHODS

Live *O. rhinoceros* adults required for this study were obtained from a culture maintained in the laboratory as described by Mini and Prabhu (1990). The 7th abdominal tergite as well as the tips of the elytra (that much part of the elytra overlying the 7th abdominal tergite) were excised from five males and five females. Specimens were then cleaned thoroughly by washing in several changes of insect Ringer and removing the adhering dirt and debris with a camel hair brush. They were then dehydrated by passing through an ascending series of alcohol (70%, 80%, 90% & 100%), 1 hour in each, and preserved in absolute alcohol until used.

Prior to SEM analysis the specimens were dried, mounted on stubs by means of double adhesive tapes and coated with a 100-200A° thick film of Gold in an IB Ion coater from EIKO, Japan. They were then subjected to scanning in a HITACHI S-530 SEM, Japan. Micrographs were taken at required magnifications. Morphological as well as morphometric details of the stridulatory apparatus were studied by analysing the micrographs.

RESULTS

Abdominal Stridulatory Structures

The 7th (penultimate) abdominal tergite of both sexes revealed a striated area on its dorsum, standing out in sharp contrast against the smooth glabrous surfaces of the adjacent segments. This zone of striations was constituted by a number of transverse, more or less parallel, striations, each consisting of a number of transverse ridges (Fig. 1; 1 & 2). This striated zone commenced a little behind the anterior margin of the tergite and extended upto its posterior margin, forming a rather scaly area towards its posterior limit. A similar scaly area along the mid-dorsal line of the tergite, the median scaly area (MSA), intercepted the striated zone into right and left halves which are rather identical. Laterally the striated zone extended about half the way from the middle line, and merged with a lateral scaly area.

Towards the anterior end of the tergite the striations were formed of highly prominent ridges which were organized into two well-distinguished zones, the zones of major striations (ZMAS), one on either side of the middle line. The rest of the striated area formed of much smaller ridges constituted the zone of minor striations (ZMIS). The largest ridges were found generally towards the upper middle part of each ZMAS as the length of the plectral ridges decreased gradually towards either side so as to merge with the median and lateral scaly areas, as also posteriorly, to merge with the zone of minor striations.

Morphometric details of *plectrum* of the male and female *O. rhinoceros* are given in Table (1). Obviously, there was significant difference between the sexes in the

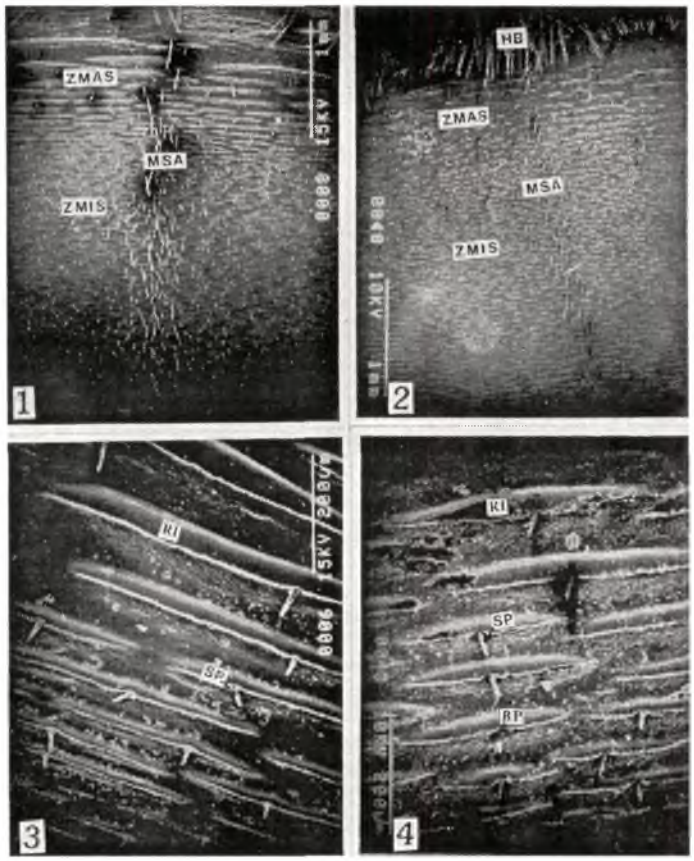

Fig. 1. [1-4]. Scanning Electron Micrographs of the dorsum of the 7th abdominal tergite in *O. rhinoceros*. (1) Mid-dorsal region of the tergite in the male (30 X). (2) Mid-dorsal region of the tergite in the female (30 X). (3) A portion of the zone of major striations (ZMAS) showing the largest ridges of the male (150 X). (4) A portion of the zone of major striations (ZMAS) showing the largest ridges of the female (150 X).
BP-Branched process; HB-Hairy belt; MSA-Median scaly area; RI-Ridge; SP-Spine; ZMAS-Zone of major striations; ZMIS-Zone of minor striations.

number of striations of the ZMAS as well as the length of its ridges. With the exception of a few large ridges of the males, the others shared a rather common breadth range, hence the differences in breadth was not significant. Thus the length: breadth ratio of these ridges remained much higher for the males than the females (17.28 and 9.4 respectively). The male ZMAS occupied a significantly greater area as well, with a significantly lesser number of ridges per unit area than in the female. However the total number of ridges in the ZMAS did not reveal significant difference between the sexes.

The zone of minor striations (ZMIS) also revealed significant difference in the length of its ridges between the sexes, though the difference in breadth was not significant.

The *plectral* area of the tergite also revealed numerous short smooth conical spine

Table 1
Morphometric details of the *plectrum* in male and female *O. rhinoceros*

	Parameter	Mean ± S.D.*		't' value for the parameter between the sexes
		Male	Female	
1.	No. of striations in the ZMAS	11.4± 1.496	6.8± 1.469	4.911 ; P < 0.01 (Significnat at 1% level)
2.	Mean length of the ridge in the ZMAS (μ)	497.535± 20.0748	283.077± 43.7570	6.2999; P < 0.05 (Significant at 5% level)
3.	Mean breadth of the ridge in the ZMAS (μ)	28.789± 2.3060	30.075± 3.8530	0.4050; P > 0.10 (not significant)
4.	Total No. of ridges in the ZMAS	93.000± 5.6569	80.000± 5.6569	0.2031; P > 0.10 (not significant)
5.	Area of the ZMAS (mm^2)	2.820± 0.3740	1.430± 0.1270	4.9588; P < 0.05 (Significant at 5% level)
6.	Length of the ZMAS (mm)	2.822± 0.1162	1.931± 0.1743	6.0040; P < 0.05 (Significant at 5% level)
7.	Breadth of the ZMAS (mm)	0.999± 0.1747	0.739± 0.0001	2.0954; P > 0.05 (not significant)
8.	No. of ridges per mm^2 of the ZMAS	33.196± 2.4090	55.9896± 1.02730	12.3089; P < 0.01 (Significant at 1% level)
9.	Mean length of the ridge in the ZMIS (μ)	241.179± 3.5220	142.049± 1.6270	36.1353; P < 0.001 (Significant at 0.1% level)
10.	Mean bredth of the ridge in the ZMIS (μ)	18.163± 0.3300	17.204± 1.0940	1.1869; P < 0.10 (not significant)
11.	Mean length of the plectral spine (μ)	37.760± 3.9030	39.570± 3.3370	0.4989; P > 0.10 (not significant)

*Based on 5 individuals

like processes (SP) bearing an 1:1 correspondence with the ridges (RI) (Fig. 1; 3 & 4). Each spine arouse from a round socket positioned at the base of a ridge on its posterior side, generally at about midway of its length. Rarely, a few of the ridges showed short branched processes (BP) of varying shapes in the place of such spines.

There was sparse distribution of delicate hairs, apparently the counterparts of the *plectral* spines, over the scaly area of the tergite, those along the median scaly area being the smallest. There was also a broad hairy belt (HB) along the membraneous anterior boundary of the tergite (Fig. 1;2); these hairs revealed a spiky appearance under higher magnification (80 x) of the SEM.

Elytral Stridulatory Structures

Unlike the *plectrum*, the elytral stridulatory structures, located towards the elytral apices, did not disclose any sexually dimorphic difference; also no difference could

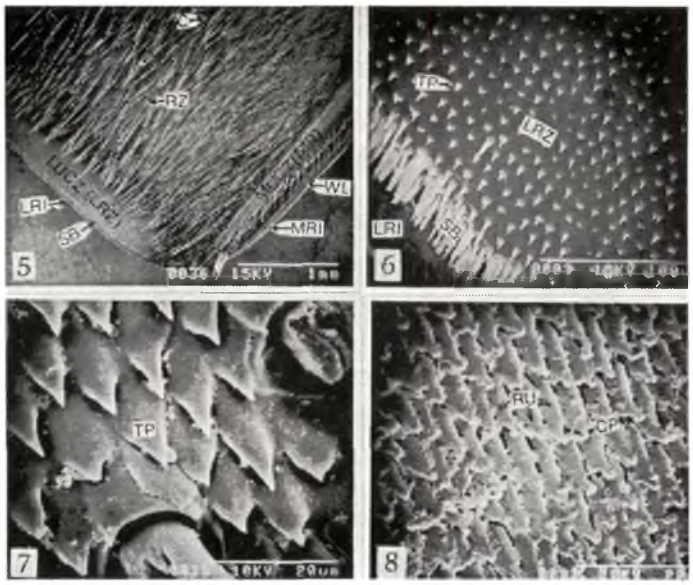

Fig. 2. [5-8]. Scanning Electron Micrographs of the inner surface of the elytral tip. (5) elytral tip showing the different distinguishable zones (30 X). (6) Lateral region of the elytral tip (500 X). (7) A portion of the apical rough zone (ARZ) (2000 X). (8) A portion of the median ridge (MR) (2000 X).
C-Concavity; CP-Conical process; LRI-Lateral rim; LRZ-Lateral rough zone; LUCZ-Lateral uncovered zone; MR-Median ridge; MRI-Median rim; MUCZ-Median uncovered zone; RU-Rod-like unit; RZ-Rough zone; SB-Spinous belt; TP-Teeth-like process; WL-Wing-lock.

be observed between the right and left elytron.

Morphological details on the inner surface of the elytral tip are shown in Fig. 2;5-8. The surface of the elytral tip, mostly covered with small hairs, revealed a rough texture forming the Rough Zone (RZ). bearing numerous regularly arranged teeth-like processes (TP). Towards the lateral and median margins of the elytron, the rough zone was uncovered forming the lateral uncovered zone (LUCZ) and median uncovered zone (MUCZ) respectively. Close to the smooth lateral rim (LRI) the teeth were longer and spine like and compactly arranged to form a spinous belt (SB). The teeth were densely aggregated at the extreme apical point, forming the apical rough zone (ARZ). The median uncovered zone was a slightly raised convex ridge, the median ridge (MR), originating near the apical point and diminishing at about the level of a concavity (C), a few centimeters above the apex, with gradual increase in width and decrease in height. Sprouting out from its surface were numerous longitudinal compactly arranged rod-like units (RU) disposed in single unit thickness. Each unit had its posterio-dorsal aspect drawn out into a slightly curved conical process (CP). These units remained mutually connected over most part of the median ridge. The portion between the median ridge and the median rim (MRI) was covered with hairs.

DISCUSSION

Ultramorphological studies on the stridulatory organs are available on a number of coleopterans representing different families, such as the chrysomelid *Lema trilineata-daturaphila* (Kogan *et al.* 1970), the curculionids *Pissodes strobi* (Harman and Harman, 1972) and *Conotrachelus* (Gibson, 1967), the scolytids *Ips* (Barr, 1969) and *Dendroctonus* (Michael and Rudinsky, 1972), and the Scarabaeids *Heliocopris bucephalus* (Narendran and Joseph, 1978) and *Heliocopris dominus* (Joseph, 1991). Majority of them possess an elytro-abdominal type of stridulatory device as found in *O. rhinoceros* though other mechanisms are also met with. Thus the scarabaeids *H. dominus* and *H. bucephalus* exhibit the hind coxa-coxal cavity mechanism (Joseph, 1991; Narendran and Joseph 1978) while it is Gula-prosternal device in some scolytids (c.f. Michael and Rudinsky, 1972) and Vertex-pronotal mechanism in *Ips* (Lewis and Cane, 1992).

During stridulation generally it is the mobile abdominal component that scrapes against the relatively stationary elytral component. The *plectrum* is hence also known as the 'scraper' or 'stridulator', and the *pars stridens*, as the 'file' or 'strigil' (Harman and Herman, 1972). However for reasons not clear, Kogan et al (1970) use the term *plectrum* to refer the elytral stridulatory component and *pars stridens* to denote the abdominal components, although the morphology of the elytral and abdominal components as well as the fundamental mechanism of sound production are similar to that of the other coleopterans mentioned here.

A stridulatory device closely resembling that of *O. rhinoceros* has been reported in *Lemma trilineatadaturaphila* (Kogan et al, 1970). On the contrary, in *Dendroctonus* (Michael and Rudinsky, 1972), *Conotrachelus* (Gibson, 1967), *P. strobi* (Harman and Harman, 1972) and *Hylobius abietis* (Selander and Jansson, 1977), the elytral structures are generally in the form of a file of transverse ridges resembling the abdominal *plectrum* of *O. rhinoceros*, while their abdominal components are formed of a few definite number of teeth or tubercles resembling the elytral spines of *pars stridens* in *O. rhinoceros*. In *Helicocopris dominus* the stridulatory structures are constituted by striations on the dorsal side of the hind coxal ridge as well as in the hind coxal cavity. It thus seems that the morphology as well as positioning of the stridulatory structures can vary even among taxonomically closely related species while at the same time more distant species may exhibit greater similarities in these respects.

A noteworthy aspect of the stridulatory apparatus of *O. rhinoceros* is the prominent elements of sexual dimorphism associated with it. The prominence of male stridulatory apparatus evidently is mainly due to certain quantitative differences pertaining to the obviously specialized part of the *plectrum* - the ZMAS - such as a greater number of striations, greater length of the ridges, and a greater area occupied by the ZMAS, all these characteristics amounting to almost double that of the female. The ZMIS also is more prominent in the males, with longer ridges.

The median ridge (MR), by virtue of its position as overlying the most specialized part of the *plectrum*, the ZMAS, and of its highly modified texture, seems to be the major (if not the only) component of the *pars stridens*. In *P. strobi* also an identical zone, possessing an orderly array of ridges was regarded as the *pars stridens* (Harman and Harman, 1972).

The present studies reveal that in *O. rhinoceros* sexual dimorphism pertaining to the stridulatory device is detectable only in the *plectrum*, as against the condition in

C. posticatus and *C. carinifer* (Gibson, 1967) in which it is the *pars stridens* which exhibits sexual dimorphism. However in *P. strobi* (Harman and Harman, 1972), *C. nenuphar* (Gibson, 1967) and *H. abietis* (Selander and Jansson, 1977), sex-related differences are manifested both in the *plectrum* as well as *pars stridens*.

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Effect of Neem Limonoids on Feeding and Reproduction of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract: The limonoid compounds deacetylnimbin, 17 - hydroxyazadiradione, gedunin, salannin and deacetylgedunin, constituents of neem tree (*Azadirachta indica*) show their effects on development, feeding and reproduction in a polyphagous insect *Helicoverpa armigera*. The neem limonoid significantly inhibited growth, longevity and fecundity when administered orally through (*Gossypium hirsutum* leaves). The reduced nutritional efficiency measures and fecundity are recorded as the consequence of post - ingested toxic effects of these compounds.

Keywords: *Helicoverpa armigera*, Neem limonoids, Feeding, Reproduction.

INTRODUCTION

Limonoids, the tetranortriterpenoids derived from the neem tree, *Azadirachta indica*, have been reported to exert a phagodeterrent effect on phytophagous insects (Saxena *et al.*, 1984; Alford *et al.*, 1987). Studies on the bioactivity of Meliaceous limonoids in insects are limited because of their low natural concentrations and complex chemical structures (Champagne *et al.*, 1992). The most prominent amongst these is azadirachtin, which either by itself or as an active ingredient of neem extracts is efficacious against nearly 200 insects and mite species (Saxena, 1989). However, its extreme structural complexity is a hindrance to its synthesis on a commercial scale. Therefore efforts in making discoveries of simpler limonoids which could be synthesized for insect control is clearly warranted. Murugan *et al.* (1993) studied the effect of neem products (neem oil and neem kernel extracts) on the nutritive and reproductive physiology of *Helicoverpa armigera*. Jeyabalan & Murugan (1995) investigated the neem limonoids on its antifeedent effect, growth inhibition and toxicity in *Helicoverpa armigera*. In the present study, we have investigated the effect of neem limonoids on food utilization and reproduction of *Helicoverpa armigera*.

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MATERIALS AND METHODS

Neem limonoids were obtained from Prof. Munetake Ishida, Central Research Laboratories, Taiyo Kayaka Co., Ltd., Japan. Each limonoid was dissolved in methanol. From the stock solutions, different concentrations were prepared by using methanol.

The leaves of *Gossypium hirsutum* (MCU-5 cultivar) were dipped in different neem limonoids and air dried. The starved (3h) third instar larvae of *Helicoverpa armigera*, were allowed to feed on various limonoids treated with cotton leaves (Busvine, 1971; Murugan *et al.*, 1995). The control leaves were treated with methanol alone. The insects (20 larvae) were maintained at 28°C ± 2°C throughout the test and checked daily until pupation. Then each one was removed from the container and placed in a clean container and observed for emergence. The days from moulting of the larvae to pupation and to adulthood were noted. Fecundity was assessed by counting the number of eggs laid during the life span in control and experimental insects. The larval and pupal durations of treated and control individuals were compared and developmental rate was also determined. Percentage of adult mortality control and experimental insects were also noted. Total protein was estimated in the ovary of moths by the method of Lowry *et al.* (1951).

The various food utilization efficiency measures were adopted from the method of Waldbauer (1968). Data were subjected to analysis of variance and the means were separated using Duncan's Multiple Range Test (Alder & Roessler, 1977) ($P < 0.05$).

RESULTS

Impact of various limonoids on the total larval duration, longevity, fecundity and adult mortality are given in Table 1. Total larval duration in control insect was 11.0 days; by deacetylnimbin treatment 12.0 days, salannin 13.0 days and deacetylgedunin treatment it was further extended to 13.4 days. Pre and Post - oviposition periods in control insects were 5.42 and 1.00 days but the above periods were reduced by salannin treatment to 4.05 and 0.84 days and further reduction of pre-and post-oviposition periods were evident (3.76 and 0.67 days) by deacetylgedunin treatment. Longevity and fecundity were minimum and adult mortality was higher by deacetylgedunin treatment than on the other limonoids treatment groups. The level of ovarian protein was significantly reduced by limonoids treatment than on control insects.

Consumption index, relative growth rate and nutritional efficiency measures (ECI and ECD) of treated individuals were reduced in comparison to those of control (Table 2).

Table 1
Effect of Neem limonoids on the development, reproduction, mortality and ovary protein parameters of *H. armigera* fed on *G. hirsutum* leaves.

Treatment	Biological parameters (days)										
	Total						Adult longevity		Fecundity	Adult	Ovary
	Larval duration	Prepupal period	Pupal period	Pre-ovi position	Ovipos-ition	Post ovi-position	Male	Female	(No. of eggs)	Mortality (%)	Protein content(mg/g)
Control	11.0^{a}	1.00^{b}	8.25^{c}	1.23^{c}	5.42^{a}	1.00^{a}	6.71^{a}	8.00^{a}	1105^{a}	0^{e}	182.6^{a}
Deacetylnimbin											
150ppm	12.00^{a}	1.26^{b}	9.13^{c}	1.54^{c}	5.00^{ab}	0.95^{a}	6.00^{ab}	7.23^{a}	1005^{b}	5^{d}	154.9^{b}
17-hydroxyaza-diradione											
30ppm	12.3^{a}	1.34^{b}	10.16^{b}	1.51^{bc}	4.86^{b}	0.90^{a}	5.83^{b}	7.00^{b}	985^{bc}	9^{c}	146.3^{bc}
Gedunin											
25ppm	12.5^{a}	1.56^{b}	11.00^{ab}	1.68^{b}	4.51^{bc}	0.84^{ab}	5.51^{bc}	6.85^{bc}	923^{c}	11^{c}	131.5^{c}
Salannin											
12ppm	13.00^{a}	1.89^{a}	11.46^{ab}	1.91^{ab}	4.05^{c}	0.79^{ab}	5.00^{c}	6.23^{c}	899^{cd}	17^{b}	96.8^{d}
Deacetyl-gedunin											
10ppm	13.4^{a}	1.96^{a}	11.87^{a}	2.00^{a}	3.76^{c}	0.67^{b}	4.85^{c}	5.81^{c}	828^{d}	25^{a}	91.7^{d}

Within column means followed by a common letter are not significantly different at 5% level by DMRT

Table 2
Effect of neem limonoids on the nutritional indices of *H. armigera* fed on *G. hirsutum* leaves

Treatment	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Control	4.72^{a}	9.57^{a}	55.31^{b}	16.65^{a}	21.46^{a}
Deacetylnimbin					
150ppm	1.02^{b}	5.01^{b}	76.19^{a}	12.08^{b}	16.22^{ab}
17-hydroxyazadiradione					
30ppm	1.00^{b}	4.96^{b}	77.23^{a}	11.92^{bc}	15.94^{b}
Gedunin					
25ppm	0.96^{b}	4.85^{b}	79.98^{a}	10.36^{bc}	14.29^{b}
Salannin					
12ppm	0.91^{b}	4.71^{b}	80.54^{a}	9.26^{bc}	12.18^{b}
Deacetylgedunin					
10ppm	0.80^{b}	4.60^{b}	81.28^{a}	7.49^{c}	10.48^{b}

Within a column means followed by a common letter are not significantly different at 5% level by DMRT

DISCUSSION

H. armigera larvae consumed less food and gained lesser weight after the limonoids treatment when compared to control. Reduced consumption of leaves in treated is likely to be the consequence of toxicity rather than cause of growth inhibition. This conclusion is well supported by the data from nutritional experiments where limonoids resulted in lower RGR and concomitant reductions in ECI and ECD. Interestingly, the RGR was significantly reduced by limonoids treatment which indicates that feeding depression was caused by behavioural effects (Jeyabalan & Murugan, 1995). Reduction of ECI and ECD confirms the deleterious effects of post - ingestive toxicity. The extended larval and pupal duration, reduced longevity and fecundity suggest that limonoids may disturb the endocrine events either to a blockage of haemolymph ecdysteroid peak, or limonoids interfere with other biochemical/physiological processes through binding to critical macromolecules is highly probable (Koul & Isman, 1991; Mordue (Luntz) *et al.*, 1986).

In the present study neem limonoids not only affected feeding and food utilization but fecundity as well. Neem limonoids greatly affected the total egg out put of *H. armigera*. Koul (1984) observed after administration of azadirachtin to *Dysdercus koenigii* (F) females a trophocyte damage. Likewise azadirachtin affected ovaries in the last instar nymphs of *Oncopeltus fasciatus* (Dorn *et al.*, 1986) and in *locusts* (Rembold *et al.*, 1987). The decreased fecundity by limonoids treatment might be due to a rapid decrease in the juvenile hormone titre and associated disturbances in oogenesis. Similar finding was noted in adult *Locusta migratoria* after azadirachtin treatment (Rembold, 1984; Rembold *et al.*, 1987).

In the present study, significant decrease in the rate of reproductive maturation caused after treatments appear to be the direct consequence of poor nutrition and also associated feeding physiology which might have influenced oogenesis. Decreased protein levels in the ovary which causes lower fecundity and oocyte development could thus be the effect of impaired vitellogenin synthesis and its uptake by the developing oocytes (Ludlum & Seiber, 1988).

Limonoids are a group of chemically related bitter tetranortriterpene derivatives predominantly present in Rutaceae and Meliaceae plants (Ishida *et al.*, 1992). Among the neem limonoids tested for food utilization and reproduction for *H. armigera*, in the present study deacetylgedunin and salannin interfered greatly on nutritional efficiency measures and fecundity of *H. armigera*. This might be due to their chemical structure and activities which played an important role (Ishida *et al.*, 1992) and particularly hydroxyl group seems to have been the main factor determining the biological activity.

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Influence of Prey Species on Feeding Response, Development and Reproduction of the Reduviid, *Cydnocoris gilvus* Burm. (Reduviidae: Heteroptera)

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Abstract: Time taken for prey recognition, paralysis and handling time of the reduviid, *Cydnocoris gilvus* was maximum on the prey insect, *Spodoptera litura* compared to *Oxya nitidula* and *Odontotermes obesus*. Basic nutrients including proteins, carbohydrates, lipids and amino acids of the prey species were estimated to assess the dietary value of the food for mass rearing. When larvae of *S. litura* were offered, fecundity and life-table parameters were maximum in relation to prey recognition and handling time.

Keywords: Handling time, Prey recognition, Prey insect, Fecundity, Life-table parameters.

INTRODUCTION

Several species of reduviids prey upon a wide range of insect species of various sizes, holding, promise as useful agents in biological control and pest management. Considering the impact of various abiotic factors and biotic factors, host nutrition plays a vital role in regulating the physiological activities of the predator. The quality and quantity of nutrients of the prey not only influences the growth rate and survival of the predator (White, 1970; Ambrose *et al.*, 1990a; O'Neil & Wiedenman, 1990), but also the fecundity and life table characteristics such as generation time, intrinsic rate of population increase (Awadallah *et al.*, 1986).

Cydnocoris gilvus has been recorded as an efficient predator of the teak skeletonizer, *Pyrausta mechaeralis* in various forest zones of Karnataka (Misra, 1975; Patil & Thontadarya, 1983). Because of the inadequate information, an insight is made to understand the feeding potential of *C. gilvus* on different prey insects namely *Spodoptera litura* (Noctuidae: Lepidoptera), *Oxya nitidula* (Acrididae: Orthoptera) and *Odontotermes obesus* (Termitidae: Isoptera) which would facilitate their augmentation under laboratory conditions.

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MATERIALS AND METHODS

A cluster of eggs was collected from Kumbakkarai (Foot hills of Kodaikanal), a region of the Pulney ranges in Southern India. The stock culture of reduviids was maintained in the laboratory for at least two generations on termites and caterpillars. For experiments, the individuals were obtained from the stock culture. Freshly hatched *C. gilvus* nymphs were transfered into a plastic insect rearing vial of 250 ml capacity (8 cm× 7 cm). Nymphs numbering twenty were maintained separately on host insects such as *S. litura*, *O. nitidula* and *O. obesus*. After the second instar, the nymphs were reared individually in a plastic vial of 8 cm × 7 cm size on the respective prey insects to avert cannibalism. Fourth instar larvae of *S. litura* were offered uniformly as prey for the entire life period of *C. gilvus*. Observations on incubation period, nymphal duration, adult longevity and fecundity of *C. gilvus* were recorded on its respective preys.

Nutrient analysis

Quantification of total carbohydrates (Dubois *et al.*, 1956), total proteins (Lowry *et al.*, 1951), amino acids (Moore & Stein, 1948) and lipids (Folch *et al.*, 1957) were carried out in the prey insects.

Life-table studies

Laboratory cultures of *C. gilvus* were maintained for two generations and used in the study of life-table parameters. When nymphs reached the final instar on different prey insects, the reduviid culture was monitored daily and newly eclosed adults were removed from the culture, paired with a member of the opposite sex and twenty five such pairs were thus observed. Data on egg laying (clutch) and the number of eggs per clutch were recorded for each female. Life-tables were constructed following the procedure described by Birch (1948). The values of X, 1x and mx were calculated using the formulae of Howe (1953).

Mean length of generation (Tc) = $\frac{Elxmx}{Ro}$

Innate capacity for increase in numbers (rc) = $\frac{\log_e Ro}{Tc}$

Corrected generation time (T) = $\frac{\log_e Ro}{rm}$

Finite rate of increase in numbers (λ) = anti e^{rm}

Weekly multiplication time = (e^{rm^7})

Doubling time = log 2/ log λ

All the experiments were carried out at 32.5± 0.5° C, 85% r.h. and 12L:12D photoperiod.

RESULTS

Time taken for host recognition, paralysis and handling time of *C. gilvus* was not uniform (Table 1). The adult reduviids recognized the larvae of *S. litura* within a shorter period of encounter than *O. nitidula* and *O. obesus* as food. Time taken for recognition (in minutes), handling time for all the prey species examined was statistically significant at 0.001% level. However, time taken for paralyzing prey such as *S. litura* and *O. nitidula* was not statistically significant, while the difference in time taken for paralyzing *S. litura* and *O. obesus* was significant (p=0.001).

Table 1
Response of *Cydnocoris gilvus* adults to selected prey species

Parameters	Prey species		
	S. litura	*O. nitidula*	*O. obesus*
Time taken for recognition (in minutes)	46.67 ± 2.94^a	41.67 ± 2.42^b	61.50 ± 1.87^c
Time taken for paralysis (in minutes)	4.08 ± 0.06^a	4.13 ± 0.09^a	3.05 ± 0.09^b
Time taken for feeding (in minutes)	70.50 ± 1.87^a	79.50 ± 1.64^b	51.32 ± 1.67^c

Values followed by similar superscripts "across a row" are not statistically different at 0.001% level.

Table 2
Developmental duration and adult longevity of *Cydnocoris gilvus* reared on selected prey species

Parameters	Prey species		
	S. litura	*O. nitidula*	*O. obesus*
Incubation period	5.33 ± 1.15^a	5.67 ± 0.58^a	5.33 ± 1.15^a
Nymphal period	62.33 ± 0.58^a	65.00 ± 1.00^b	68.00 ± 1.00^c
Longevity			
Male	34.33 ± 1.53^a	31.00 ± 1.00^b	20.33 ± 0.58^c
Female	43.00 ± 1.00^a	39.00 ± 1.00^b	27.00 ± 1.00^c
Fecudity	89.00 ± 2.65^a	72.00 ± 1.73^b	45.30 ± 1.15^c

Values followed by similar superscripts "across a row" are not statistically different at 0.05% level.

Irrespective of the prey insects, egg incubation period of *C. gilvus* lasted for 5.3 to 5.7 days. However, the shortest and longest nymphal period of the predator was recorded when fed with *S. litura* and *O. obesus* respectively (Table 2). Also longevity of both the sexes of *C. gilvus* was statistically higher on *S. litura* than on *O. nitidula* and *O. obesus*. In general, the longevity of male reduviids was comparatively minimum than female insects reared on all the prey species. In relation to prey recognition and handling time, fecundity was also maximum when the larvae of *S. litura* were offered than adults of *O. nitidula* and *O. obesus* as food.

Biochemical correlates of all the host species were identified, since prey recognition and reproductive potential of predators depend on the nutrients of prey species. Primary nutrients such as total proteins, total carbohydrates, total lipids and total amino acids were relatively higher in quantity in the larvae of *S. litura* than in the other prey species (Table 3). The level of total proteins in *O. obesus* and *O. nitidula* was not statistically significant. However, a significant difference ($p = 0.001$) was observed in the protein level of *S. litura*. In addition, significant difference in the level of carbohydrate, lipid and amino acids were recorded in all the prey species.

Table 3
Nutrient diversity of prey species of *Cydnocoris gilvus*

Prey species	Proteins (mg/g)	Carbohydrates (mg/g)	Lipids (mg/g)	Amino acids (mg/g)
O. obesus	9.103 ± 0.052^a	7.620 ± 0.141^a	5.517 ± 0.512^a	6.068 ± 0.124^a
O. nitidula	8.982 ± 0.150^a	7.037 ± 0.096^b	12.483 ± 0.360^b	5.780 ± 0.092^b
S. litura	10.350 ± 0.268^b	8.570 ± 0.042^c	14.215 ± 0.358^c	8.055 ± 0.103^c

Values followed by similar superscripts "across a column" are not statistically different at 0.001% level.

Table 4
Mean length of generation, finite rate of increase in numbers and doubling time of *Cydnocoris gilvus* when reared on selected prey species.

Parameters	Prey species		
	S. litura	*O. nitidula*	*O. obesus*
Net reproductive rate (Ro)	31.90	20.94	12.88
Mean length of a generation (TC)	72.62	76.36	80.55
Innate capacity for increase in numbers (rc)	0.048	0.040	0.030
Corrected rm	0.060	0.052	0.044
Corrected generation time (T)	57.72	58.50	58.09
Finite rate of increase in number (λ)	1.0618	1.0534	1.0449
Weekly multiplication	1.522	1.439	1.361
Doubling time (Days)	11.58	13.32	15.76

The net reproductive rate of *C. gilvus* was maximum on *S. litura* (31.90 days), compared to *O. nitidula* (20.94 days) and *O. obesus* (12.88 days) (Table 4). In addition, a mean length of generation time of the predator was shorter when fed on *S. litura* (72.62 days) than on *O. nitidula* (76.36 days) and *O. obesus* (80.55 days). Besides, the innate capacity for increase in numbers was also higher on *S. litura* than on *O. nitidula* and *O. obesus*. Consequently, the intrinsic rate of population increase was highest on *S. litura*. Based on the rm value, host suitability and age specific fecundity (mx) decreased in the order of *S. litura* > *O. nitidula* > *O. obesus*. Survivorship and natality patterns of *C. gilvus* under three regimens were presented in Figure 1. Corresponding to the intrinsic rate, population doubling time increased from 11.58 days on *S. litura* to 13.32 and 15.76 days on *O. nitidula* and *O. obesus* respectively.

DISCUSSION

Variation in the time taken for prey recognition, and for attacking the various prey by *C. gilvus* was statistically significant. Among the prey insects, the time taken for rec-

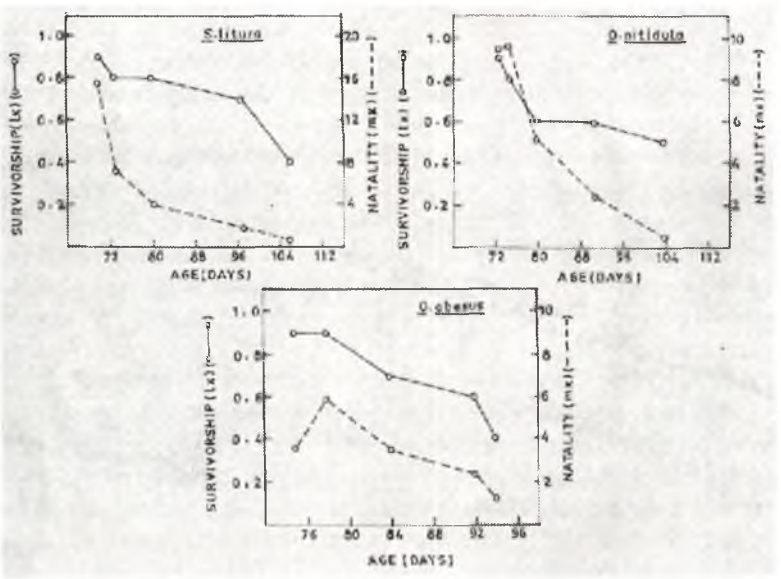

Fig. 1 Survivorship (lx) and natality (mx) patterns for *C. gilvus* cohorts at different prey regimens.

ognizing *O. obesus* was maximum due to the feeding stress developed by the small size and the odour of the formic acid secreted by the prey. Similarly, prolonged feeding time was observed in the nymphal stages of *Rhynocoris marginatus* when fed on *O. obesus* and *Camponotus compressus* (Ambrose *et al.*, 1990b). Small body size and swift running is the main prey defence for thrips (Lawrence & Watson, 1979; Crocker & Whitcomb, 1980) rendering the prey unacceptable to adult *Geocoris ochropterus*. Multiple attack on *O. obesus* was common and hence the time needed to avail the prey can significantly influence the predation rate (Wiedenmann & O'Neil, 1991). Prey consumption and predation rate of *Orius maxidentex* and *Carayonocoris indicus* differed depending on the developmental stage and sex of *Thrips palmi* and *Haplothrips ganglbaueri* (Sureshkumar & Ananthakrishnan, 1984). Maximum resting period was noticed after feeding the eggs of *Heliothis virescens* by the lygaeid predator, *Geocoris punctipes*. The resting period is associated with decreased water loss (Cohen, 1982), decreased oxygen consumption and metabolic rates (Cohen, 1984; 1985). Time taken for paralyzing prey such as *S. litura* and *O. nitidula* was not significant, possibly due to the injection of increased concentration of the toxin (De Clercq 1993).

Variations in the quantity of nutrients of prey species appear to have considerable effect on the feeding efficiency, reproductive potential of the predators (Beddington, 1975). The shortest nymphal period of the predator *C. gilvus* was recorded when reared on *S. litura* and *O. nitidula* which might be due to the minimum stress developed during predation on less number of preys due to their comparatively larger size with richer body tissue. Eggs, I and II instar larvae of beet army worm proved to be inadequate food for *Podisus maculiventris*, while the IV instar provided adequate nutrients for completing the life cycle of the predator (De Clercq & Degheele, 1994).

Pre-reproductive delay and reproductive potential such as fecundity, per cent hatch-

ability of insect predators are determined by the nutrient composition of the prey species (Fuller, 1988). Egg laying potential, hatching success and longevity of adults were maximum on *S. litura* than on other prey species due to higher content of primary nutrients. Reduced level of pre oviposition delay, oviposition period and fecundity of *Menochilus sexmaculatus* was observed on the eggs of ants than on its natural host, *Aphis craccivora* (Agarwala & Choudhuri, 1995). Ambrose *et al.* (1990a) observed that the oviposition period of *R. marginatus* was maximum when fed with *O. obesus*. However, shorter oviposition period of the anthocorid, *Cardiastethus nazarenus* was recorded on the eggs of *Icerya purchasi* than *Lepidosaphes beckii* and *Ceratitis capitata* (Awadallah *et al.*, 1976). A wider variation in the fecundity of *M. sexmaculatus* was reported owing to feeding on the adults of *M. persicae* and *Schizaphis graminum* (Haque & Islam, 1982; Campbell *et al.*, 1980). In the present investigation, shorter duration of adult male of *C. gilvus* than female insects on all prey species was agreeable with the earlier reports of Saharia (1980) and Gautam (1989).

All life-table statistics varied with the prey species. Natality and survivorship curves reflected the higher net reproductive rate (Ro) of *C. gilvus* on *S. litura* than on other prey species. The Ro of the anthocorid, *Lyctocoris campestris* varied with maximum values on pyralids and minimum values on *Trichoplusia ni* (Arbogast *et al.*, 1983). Similarly, Awadallah *et al.* (1986) observed that the female survivorship and fecundity of *Xylocoris flavipes* was maximum on *Tribolium castaneum* compared to *Oryzaephilus surina* as prey species. Hence, considerable attention is needed to select the appropriate prey species for augmenting the insect predators in pest management practice.

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The Nutritive Effects of Sinafort$^{(R)}$ - B on *Bombyx mori* L.

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Abstract: The present investigation reports the effect of Sinafort$^{(R)}$ - B, a formulation of thiamin HCl, riboflavin, pyridoxine HCl and nicotinamide, supplementation to feed on *Bombyx mori* L. Lower concentrations (0.16 and 0.32%) of the chemical increased the growth of larvae, pupae and adults; enhanced the cocoon characters and increased the reproductive potential of the silkworm and on the other hand, higher concentrations had deleterious effects on these parameters.

INTRODUCTION

Vitamins are organic compounds required in small quantities for the maintenance of vital metabolic functions. The vitamin B complex is a large group of water soluble vitamins which function as coenzymes or precursors of coenzymes.

Significant developments in the research on silkworm nutrition started with the formulation of artificial diets. Ito (1978) reviewed the information in this field. It has been demonstrated that biotin, cholin, folic acid, inositol, nicotinic acid, pantothenate, riboflavin and thiamin are the essential vitamins for the growth and development of *B. mori* larvae (Horie and Ito,, 1963; Horie *et al.*, 1966) and the minimal optimal levels of these vitamins in the diet have also been determined (Horie and Ito, 1965; Horie *et al.*, 1966). According to Horie and Watanabe (1980), the specific dose requirements of various vitamins suggest their specificity for different metabolic functions.

The present investigation reports the effect of Sinafort$^{(R)}$ - B, a vitamin-B mixture, on the growth and development of the indigenous *Nistari* race of the silkworm, *B. mori*.

MATERIALS AND METHODS

Newly hatched larvae of *B. mori* (race - Nistari) were reared up to the first day of their third instar on finely chopped mulberry (*Morus alba* L.) leaves. Sinafort$^{(R)}$ - B, a Vitamin-B complex tablet manufactured by the Ibn Sina Pharmaceutical Industry Ltd., Gazipur, Bangladesh, was bought from the local market. Each tablet has the following composition: Thiamine HCl (B_1)BP 5 mg, Riboflavin (B_2) BP 2 mg, Pyridoxine HCl

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(B_6) BP 2 mg and Nicotinamide BP 20 mg. Four concentrations of these B-vitamins, e.g. 0.16, 0.32, 0.64 and 1.28% were made by mixing requisite amounts of the tablets in distilled water.

Fresh mulberry leaves were dipped in these concentrations and were fed to silkworms from their first day of the third instar until spinning. A batch of control worms was raised on mulberry leaves dipped in distilled water only. The improved methods of silkworm rearing as recommended by Krishnaswami (1978) were followed. The experiments were repeated thrice, each with 150 worms.

The mature larval, pupal and adult weight were individually determined on an electric balance, and the cocoon characters, viz. whole cocoon and shell weight were also recorded. The larval and pupal periods were recorded. The females were observed for their fecundity and fertility.

The growth indices of treated *B. mori* were computed as follows:

Index name	Formula	Reference
1. Larval/pupal/adult weight Index	Average larval/pupal/adult weight on treated food / Average larval/pupal/adult/weight on standard food	Joshi (1985)
2. Pupal Index	Average pupal weight on test plant / Average pupal weight on standard plant	Prasad and Bhattacharya (1975)
3. Larval-pupal Index	Average larval+pupal period on standard plant / Average larval+pupal period on test plant	-do-
4. Silk Index	Average cocoon shell weight on treated food / Average cocoon shell weight on standard food	Joshi (1985)
5. Oviposition Index	Average number of eggs laid on test plant / Average number of eggs laid on standard plant	Desmukh *et al.* (1977)

The investigation was conducted at 30 ± 1°C and 83 ± 3% R.H.

Table 1
Effect of Sinafort$^{(R)}$ - B on the weight (mg) and weight index of larva, pupa and adult of *Bombyx mori* L.

Concentration(%)	Stages: Larvae m^{-} ± SD	Larval weight Index	Pupae m^{-} ± SD	Pupal weight Index	Adults m^{-} ± SD	Adult weight Index
0(control	1967.25 ± 139.07	–	691.65 ± 48.40	–	261.35 ± 33.79	–
			(930.20 ± 68.49)**	–	(586.00 ± 71.32)	–
0.16	2240.30 ± 173.92	1.1388	717.30 ± 41.98	1.0371	274.30 ± 43.66	1.0496
			(961.10 ± 78.54)	(1.0364)	(640.71 ± 47.04)	(1.0934)
0.32	2367.65 ± 120.21	1.2035	806.00 ± 63.06	1.1653	288.00 ± 33.18	1.1019
			(1063.00 ± 92.18)	(1.1428)	(707.68 ± 84.19)	(1.2076)
0.64	1958.15 ± 118.47	0.9954	680.70 ± 59.68	0.9842	213.45 ± 53.14	0.8167
			(892.45 ± 78.86)	(0.9594)	(524.14 ± 68.11)	(0.8944)
1.28	1854.25 ± 102.40	0.9426	650.00 ± 58.26	0.9398	186.68 ± 57.18	0.7143
			(869.30 ± 90.19)	(0.9345)	(434.56 ± 78.49)	(0.7416)
F-ratio	54.99 (P<0.001)		23.37 (P<0.001)		15.44 (P<0.001)	
			14.33 (P<0.001)		16.62 (P<0.001)	
CD at 5%	266.12		111.95		36.95	
			(182.81)		(229.48)	

* Mean ± standard deviation

**Figures in parentheses show corresponding values in females

RESULTS AND DISCUSSION

The growth of *B. mori* larvae, pupae and adults were significantly influenced when the worms were reared on Sinafort$^{(R)}$ - B supplemented diet (Table 1). The highest and lowest growths were observed at 0.32 and 1.28% of the chemical respectively and the order of growth was 0.32 > 0.16 > 0 (control) > 0.64 > 1.28% Sinafort$^{(R)}$ - B.

The cocoon characters of *B. mori* were varied following vitamin supplementation (Table 2). The shell-ratios (%) and silk-indices were improved at 0.16 and 0.32%, and higher doses of the chemical (0.64 and 1.28%) produced deleterious effects on these parameters.

The larval and pupal periods of treated *B. mori* were significantly reduced at 0.32, 0.64 and 1.28% Sinafort$^{(R)}$ - B (Table 3).

The reproductive potential of the treated female moths was significantly increased due to treatments with Sinafort$^{(R)}$ - B (Table 4). The highest and lowest mean oviposition and fertility of *B. mori* females were obtained at 0.16 and 1.28% respectively.

In general, lower concentrations of Sinafort$^{(R)}$ - B improved the growth and development of *B. mori* and on the other hand, the higher concentrations had deleterious effects on the worms.

Improved growth and development of various insects following vitamin supplementation have been recorded by several workers. Ito (1961a,b) observed improve-

Table 2
Effect of Sinafort(R) - B on the cocoon characters of *Bombyx mori* L.

Concentration (%)	Characters: Cocoon weight(mg) m⁻ ± SD	Shell weight (mg) m⁻ ± SD	Shell-ratio (%) m⁻ ± SD	Silk Index
0 (control)	797.70 ± 50.04	106. 05 ± 14.17	13.29 ± 2.13	-
	(1050.27 ± 68.90)	(120.07 ± 09.85)	(11.43 ± 1.06)	-
0.16	834.00 ± 43.53	116.70 ± 14.38	13.99 ± 1.68	1.1004
	(1107.65 ± 98.53)	(143.55 ± 14.97)	(12.96 ± 1.39)	(1.1956)
0.32	927.45 ± 61.45	121.45 ± 12.44	13.10 ± 1.62	1.1452
	(1218.05 ± 93.24)	(155.05 ± 14.01)	(12.73 ± 1.59)	(1.2913)
0.64	784.20 ± 56.99	103.50 ± 07.34	13.20 ± 1.34	0.9760
	(1011.85 ± 77.99)	(119.40 ± 11.62)	(11.80 ± 1.52)	(0.9944)
1.28	740.15 ± 63.31	90.15 ± 11.15	12.18 ± 1.38	0.8500
	(984.80 ± 92.53)	(115.50 ± 09 67)	(11.73 ± 1.74)	(0.9619)
F-ratio	31.01 ($P < 0.001$)	19.96 ($P < 0.001$)	3.84 ($P < 0.05$)	
	(26.94 ($P < 0.001$)	(41.24 ($P < 0.001$)	3.76 ($P < 0.05$)	
CD at 5%	114.24	24.99	3.37	
	(174.62)	(24.93)	(3.02)	

ment in the growth and development of *B. mori* larvae following ascorbic acid supplementation. Ito and Arai (1965) reported that the absence of ascorbic acid in the diet of first and second instar *B. mori* larvae resulted in poor growth and development from third instar onwards. The role of ascorbic acid in the development of silkworm larvae has also been indicated by Gomma *et al.* (1977). Yosuhiro and Sholchi (1971) recorded that the silkworm growth was reduced when the diet was lacking in folic acid.

Khalequzzaman and Ansary (1982), and Khalequzzman and Mannan (1982) also recorded enhanced growth in *B. mori* when the worms were reared on artificial diets enriched with ascorbic and folic acids. Rahman *et al.* (1990) observed that *B. mori* reared on ascorbic acid enriched mulberry leaves produced more eggs than did the untreated females. Improvements in commercial characters of *B. mori* due to ascorbic acid supplementation have also been recorded by Sengupta *et al.* (1972) and Madhu Babu *et al.* (1992).

Ito and Arai (1965) observed that the weight of fourth instar *B. mori* larva decreased significantly from 1.98g when the young worms were fed on 1.5% ascorbic acid to 1.83g with 4.5% ascorbic acid and similar decreasing trends in the weight of cocoon and pupae were noted. High levels of riboflavin have been shown to be detrimental to silkworm larvae (Horie *et al.*, 1966). Khan and Faruki (1990) investigated the effects of supplementation of para-Amino Benzoic Acid on *B. mori*. The vitamin slightly increased larval and pupal growth at lower concentrations (1 and 10 ppm) but it produced detrimental effects on adult growth, pupation and adult emergence.

The growth of house cricket nymphs were improved following vitamin K_1 supplementation (McFarlane, 1976). In the vitamin-B complex, thiamin, riboflavin, nicotinic

Table 3
Effect of Sinafort$^{(R)}$ - B on the developmental periods (days) of *Bombyx mori* L.

Concentration (%)	Larval period $m^- \pm SD$	Pupal period $m^- \pm SD$	Total developmental period	Larval-pupal Index
0 (control)	17.16 ± 0.62	10.11 ± 0.83	27.27	–
0.16	18.10 ± 0.78	10.24 ± 0.61	28.34	1.0392
0.32	16.25 ± 0.40	9.42 ± 0.73	25.67	0.9413
0.64	16.67 ± 0.64	9.60 ± 0.66	26.27	0.9633
1.28	16.90 ± 0.57	9.92 ± 0.65	26.82	0.9835
F-ratio	2.68 ($P < 0.05$)	2.45 ($P < 0.05$)		
CD at 5%	1.59	1.37		

Table 4
Effect of Sinafort$^{(R)}$ - B on reproductive potential of *Bombyx mori* L.

Concentration (%)	Fecundity $m^- \pm SD$	No. of females	Oviposition Index	Fertility(%) $m^- \pm SD$	No. of eggs
0 (control)	426.11 ± 26.70	45	–	96.27 ± 1.39	19175
0.16	435.60 ± 52.06	45	1.0223	97.83 ± 1.21	19602
0.32	452.15 ± 22.91	45	1.0611	98.50 ± 1.03	20347
0.64	400.20 ± 47.17	45	0.9392	94.25 ± 4.94	18009
1.28	383.60 ± 68.35	45	0.9003	90.62 ± 2.85	17262
F-ratio	2.97 ($P < 0.05$)			12.64 ($P < 0.001$)	
CD at 5%	103.79			4.31	

acid, pantothenic acid, pyridoxine and cholin chloride were noted to be essential for the growth of fruit fly larvae, *Dacus cucurbitae* (Srivastava and Pant, 1977). The requirement for niacin as a vitamin by *B. mori* is well-demonstrated (Horie and Ito, 1963, 1965).

The results of the present work corroborates with the findings presented in the preceding paragraphs. It may be suggested that lower concentrations of Sinafort$^{(R)}$ - B, *i.e.*, B-Vitamins may be used by the rearers to increase the economic characters of the indigenous poor silk-yielding varieties, like *Nistari*.

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Chromosomal Studies of Three Species of Indian Coleoptera

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Abstract: Chromosomes of three species of beetles: *Alcides signatus* Boh. (2n = 26), *Paralixus brachyrrhinus* Boh. (2n = 20) (Curculionidae) and *Hydrophilus kasmeriensis* Redtenbacher (2n = 30) (Hydrophilidae) were analysed for their number, morphology and behaviour. The sex chromosome mechanisms in three species were determined and their relationships among confamilial and congeneric species have been discussed.

Keywords: Karyology, Coleoptera, *Alcides signatus*, *Paralixus brachyrrhinus*, *Hydrophilus kasmeriensis*.

INTRODUCTION

The coleopterans are considered to be one of the most successful order of the animal kingdom due to its great adaptability to exploit almost all the ecological niches of the environment. There are near about 350,000 described species of beetles (Richards and Davis 1977). The utility and scope of the study of coleopteran chromosomes from the evolutionary stand point have been well recognized by a number of workers (Petitpierre *et al.* 1993; Smith 1962; Suomalainen 1961; Virkki, 1984) and it is still meagre in comparison to their huge number. With a view to extend our knowledge on coleopteran chromosomes we have undertaken the chromosomal studies of three species of karyologically unknown Indian Coleoptera viz., *Alcides signatus*, *Paralixus brachyrrhinus* and *Hydrophilus kasmeriensis* belonging to two different families.

MATERIALS AND METHODS

The species under the present investigation are listed in Table 1 along with their time of collection, host/source, place of collection, diploid values and chromosomal formulae. The adult males served as the material for the present investigation. The males were sacrificed and their testes were dissected out in 0.67% normal saline. The testicular material after pretreatment in 1% hypotonic solution of sodium citrate for 15 minutes was fixed in 1:3 aceto-alcohol mixture for 20minutes. The material was squashed in 50% acetic acid, passed through different grades of alcohol, air dried and stained with Giemsa and then mounted in D.P.X.

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Table 1
Three species of Coleoptera showing their time of collection, host/source, place of collection, 2n and chromosomal formulae.

Family and species	Time of collection	Host/Source	Place of Collection	2n	Chromosomal formulae
Family - Curculionidae					
Sub-family-Alcidinae (Gymnetrinae)		*Impomea*	Bank of	26	12 AA + XY
Alcides signatus (Boheman)	August	*pistulus*	river, Kathajori, Cuttack		
Sub-family-Cleoninae					
Paralixus brachyrrhinus (Boheman)	April	*Amaranthus viridis*	-do-	20	9 AA + Xyp
Family - Hydrophilidae					
Sub-family-Hydrophilinae					
Hydrophilus kasmeriensis (Redtenbacher)	June	Pond water	Cuttack	30	14 AA + XY

RESULTS

Alcides signatus: The spermatogonial metaphase count reveals the presence of 26 chromosomes (24A + XY). Among 12 pairs of autosomes, there are 8 pairs of metacentrics, 2 pairs of submetacentrics and 2 pairs of acrocentrics. The sex chromosomes (X and Y) are of same size having same morphology (sm-type) (Fig. 1 and 2). The metaphase I shows 13 pairs of monochiasmatic bivalents (Fig. 3). The TCL was determined to be 17.60 μm.

Paralixus brachyrrhinus: The spermatogonial complements show 20 chromosomes (18A + Xyp). Among the autosomes there are 7 pairs of metacentrics and the rest two pairs are acrocentric chromosomes. The sex chromosomes are also a-type (Fig. 4 and 5). The metaphase I shows 10 pairs of bivalents having a single chiasma per bivalent. The sex chromosomes Xyp show parachute association (Fig. 6). The genome length was measured to be 14.80 μm.

Hydrophilus kasmeriensis: The diploid chromosome number ascertained from the spermatogonial metaphase 2n = 30 (28A + XY). Among 14 pairs of autosomes there are 8 pairs of metacentrics, one pair submetacentric and the remaining 5 pairs are acrocentrics. X and Y are of same size having same morphology (metacentrics) (Fig. 7 and 8). It shows XY type of sex chromosome mechanism. The metaphase I shows 15 pairs of bivalents having single chiasma per bivalent (Fig. 9). The genome length was calculated to be 34.40 μm.

Table 2
Comparative morphometric data of the chromosomes of three species of Coleoptera

SPECIES		CHROMOSOME NUMBERS															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	X	Y
Alcides	L^R	11.36	9.10	7.95	7.95	7.95	7.95	6.82	6.82	6.82	6.82	6.82	6.82			6.82	6.81
signatus	I^C	40.00	50.00	40.20	42.80	28.50	28.50	50.00	50.00	50.00	50.00	–	–			33.30	33.30
Paralixus	L^R	13.51	10.81	10.81	10.81	10.81	10.81	8.11	8.11	8.11						8.11	5.40
brachyrrhinus	I^C	40.00	50.00	50.00	50.00	50.00	50.00	50.00	–	–						–	–
Hydrophilus	L^R	9.88	9.30	8.13	6.97	6.97	6.97	6.97	6.39	5.82	5.82	5.82	5.82	5.24	5.24	4.66	4.65
kasmeriensis	I^C	45.60	37.50	42.80	50.00	50.00	50.00	50.00	36.30	–	–	50.00	–	–	–	50.00	50.00

L^R = Relative length
I^C = Centromeric index

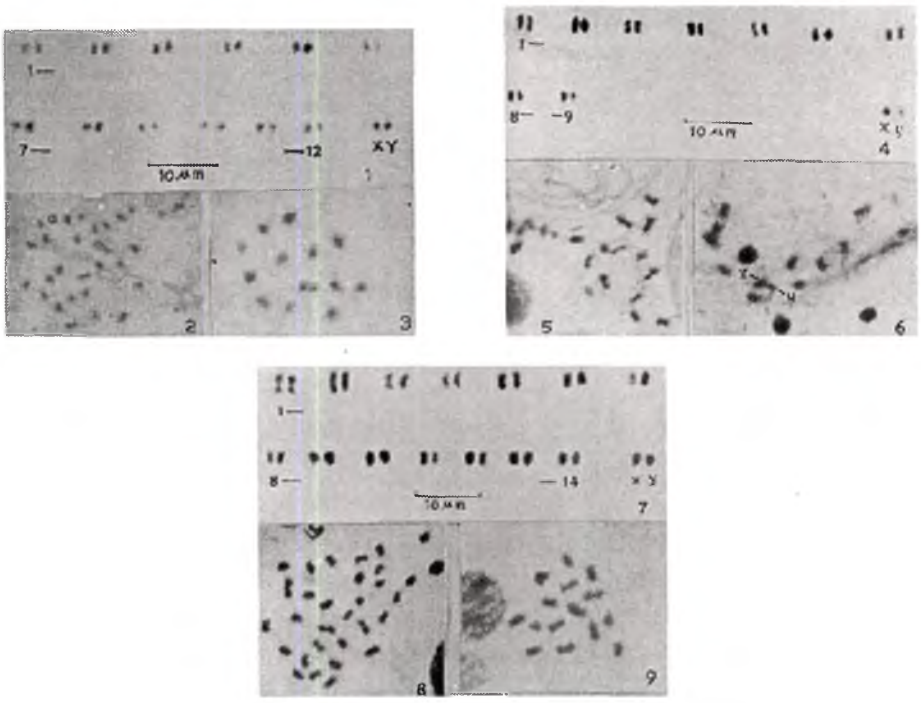

Fig. 1. Karyogram of male *Alcides signatus* Fig. 2. Spermatogonial metaphase of *A. signatus* Fig. 3. Metaphase I of *A. signatus* Fig. 4. Karyogram of male *Paralixus brachyrrhinus* Fig. 5. Spermatogonial metaphase *P. brachyrrhinus* Fig. 6. Metaphase I of *P brachyrrhinus* Fig. 7. Karyogram of male *Hydrophilus kasmeriensis* Fig. 8. Spermatogonial metaphase of *H. kasmeriensis* Fig. 9. Metaphase I of *H. kasmeriensis*

DISCUSSION

The family Curculionidae is one of the largest families of Coleoptera comprising of about 35,000 species. Although extensive chromosomal survey has been made on the foreign species of this family, the curculionids in India remain cytologically unexplored so far (Sharma 1983). The "modal haploid number" for this family is 11. The evolution of chromosome number in Curculionidae is more directed towards an increase in number, rather than the reverse. Majority of the curculionids carry the basic Xyp or Xy sex chromosome system (Yadav 1972).

In the sub-family Gymnetrinae so far seven species are cytologically known (Dasgupta 1977). A modal chromosome number could not be determined because of variation of diploid numbers among the species and the 2n varied from a minimum of 28 to a maximum of 40. The sex chromosome mechanism is either Xy or Xyp. The present species *Alcides signatus* has a lower diploid value (2n = 26) and homomorphic sex chromosomes (Table 2).

The sub-family Cleoninae is cytologically known through nine species. The diploid chromosome number varies from a minimum of 30 in *Lapyrus* spp to 46 *Lixus maculatus* (Yadav and Pillai 1975). The modal number is not known and the family is cytologically unstable (Yadav and Pillai 1975). The presently worked out species *Par-*

alixus brachyrrhinus has a lower diploid value (2n = 20) having Xyp sex chromosome mechanism. However, this species follows the most frequent and primitive number of Coleoptera *i.e.*, 2n = 20 (9AA + XYp) with Xyp sex mechanism.

In the family Hydrophilidae so far 10 species are cytologically known (Dasgupta 1977). In this family 2n varies from 18 to 30 and the sex bivalent show Xyp association with some exceptions as in *Hydrophilus olivaceus* (2n = 29) where XO sex mechanism was reported (Dasgupta 1977). In the present species *Hydrophilus kasmeriensis* the diploid number is high (2n = 30) having XY type sex mechanism (Table 2). There are three such species where the diploid values were reported to be 2n = 30. However, in the later category of species the sex chromosome mechanism is Xyp not XY type as in the present species *H. kasmeriensis*.

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Density, Biomass And Nutrient Concentration of Aboveground Insects in a Temperate Grassland Community

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Abstract: Population numbers of aboveground insects, biomass and concentrations of three elements in the insects and in vegetation in a temperate grassland at Naukuchiatal, Nainital in between February 1986 to January 1988 revealed that mean net aboveground primary production was 345 g $m^{-2}yr^{-1}$. Herbivores were the dominant group represented by Orthoptera, Homoptera, Heteroptera, Coleoptera and Lepidoptera. Maximum density and biomass were 9.8 m^{-2} and 333.8 mg m^{-2}, respectively. Mean secondary net production due to the herbivores was 586.8 mg m^{-2} yr^{-1}. Highest concentration of Na was recorded in predators and no significant differences in K and Ca were recorded in trophic levels.

Keywords: Density, Biomass, Insects, Concentration of nutrients, Grassland, India

INTRODUCTION

Grasslands are well established ecosystems for supporting not only large herbivores, but also smaller herbivores like insects. Detailed studies on population density, biomass have been done in both temperate and tropical grasslands (Wiegert, 1965; Gyllenberg, 1969; Riegert *et al.* 1974; Kaushal & Vats, 1984a). Nutrient concentration of trophic components of insect fauna have also been reported in forest floor arthropods (Reichle *et al.* 1969; Werner, 1983); orthopteran population in a grassland ecosystem (Van Hook, 1971); litter fauna in a white pine ecosystem (Cornaby, 1973); plant arthropods in the taiga ecosystem (Werner, 1983).

But apart from our earlier preliminary report (Kaushal & Joshi, 1988), there are no such studies on temperate grasslands in India. The present investigation is an attempt to understand some structural and functional aspects of a grassland community in Kaumaun Himalayas. We studied vegetational composition, density and biomass of insect fauna, secondary net production and concentration of Na, Ca, and K in the different trophic levels in a grassland community from February 1986 to 1988.

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MATERIALS AND METHODS

The study site is a 5 ha area of grassland at Naukuchiatal, about 26 km from Nainital at an altitude of 1500 m above sea level (29° 21′N 70° 35′E) with an annual rainfall of 1819 mm. The maximum temperature ranged from 12° C (Feb.) to 25.5° C (June) in 1986-87; and 12.5° C (Feb.) to 25.8° C(June) in 1987-88. The minimum temperature ranged from 8.4° C (Dec.) to 21.5° C (June) in 1986-87; and 8.4° C (Feb.) to 21.5° C (June) in 1987-88 (Fig. 1). The year is divided into three distinct seasons, viz. rainy season (second fortnight of June to October), winter (November–February) and summer (March–June).

The above–ground plant biomass was determined monthly through 50 × 50 cm harvest plots. Five visually homogeneous areas were selected at random on each collection date. Above–ground primary production was determined by through–peak analysis of live plus recent dead material (Singh *et al.* 1975). In this method, the sum of successive positive changes in the live biomass and some of positive changes in the standing dead biomass when occurred concurrently are totalled.

Population density was estimated by the removal trapping method. For this purpose, a cage with each side of 1 m^2 area was constructed, with an entrance of 80 × 40 cm on one side 10 cm above ground level. Wire gauze of 5 meshes per cm was fixed on all sides except on the side facing the ground. This mesh size of wire gauze prevented escape of insects from the cage. Random sampling from 5 different areas at 15 days interval was done, except in winter when it was done monthly, keeping in mind that the cage did not disturb the tips of the vegetation. The trapped insects were killed in jars containing ethyl acetate.

Collected insects were oven-dried to constant weight (60° C for 72 h). Each specimen was weighed in a single pan electric balance (0.01 mg accuracy) for biomass estimates.

Secondary production was calculated using Wiegert's (1965) expression:

$$P = S\sum_{i=2}^{n}\frac{(Ni+Ni-1)}{2}(Wi-Wi-1)$$

where, Ni is the number of herbivores present at time i, Wi is the mean weight per insect at time i; n is the sampling period; S is the weight of standing crop at time i = 1. It was assumed that Ni $\leq$ Ni-1 and Wi $\geq$ Wi-1. When Wi was less than Wi-1, production was considered as zero.

Collected insects were separated into individuals occupying different trophic levels, i.e. herbivores, omnivores, saprophytes and parasites, on the basis of their food habits. Insects and dried samples of the vegetation were ground and re–dried at 60° C for 24 h. These samples were then digested in a mixture of concentrated H_2So_4, HNO_3 and HCL (10:3:1) by heating 100 mg of sample in 20 ml of the tri–acid mixture on a hot sand bath till the mixture turned colourless. After digestion, distilled water was added to make up the mixture to 100 ml. Na, K, Ca were determined by flame photometry (Jackson, 1958) using standard solutions of NaCl, KCl and $CaCO_3$ for calibration.

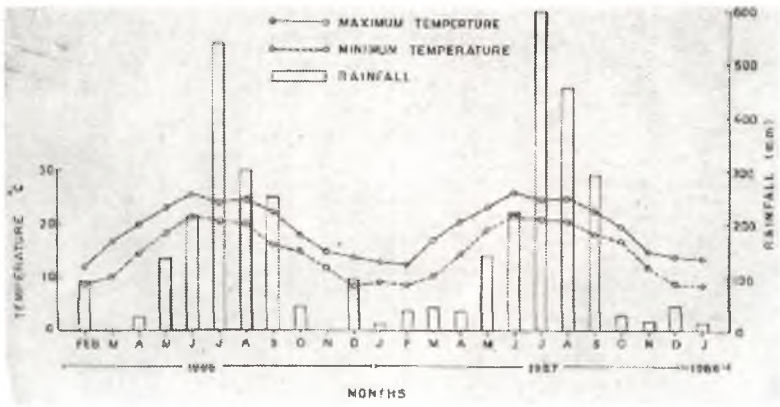

Fig. 1.

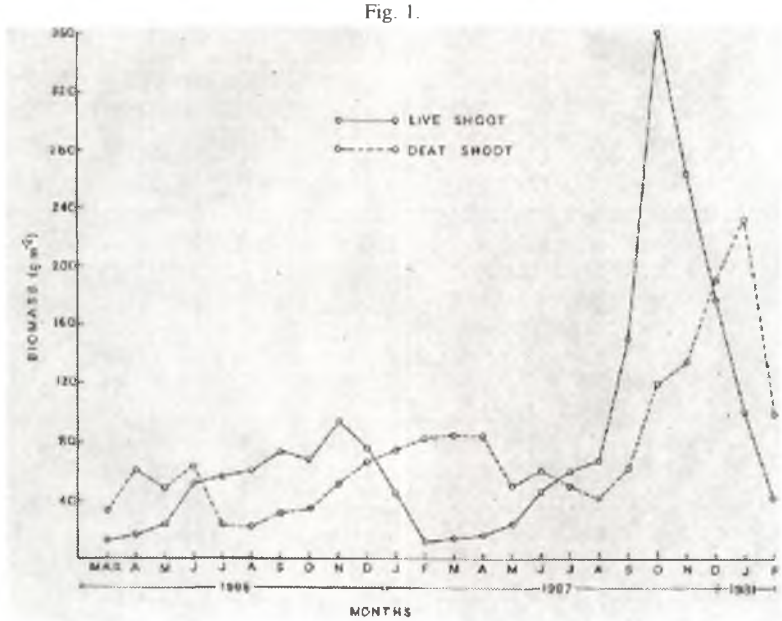

Fig. 2.

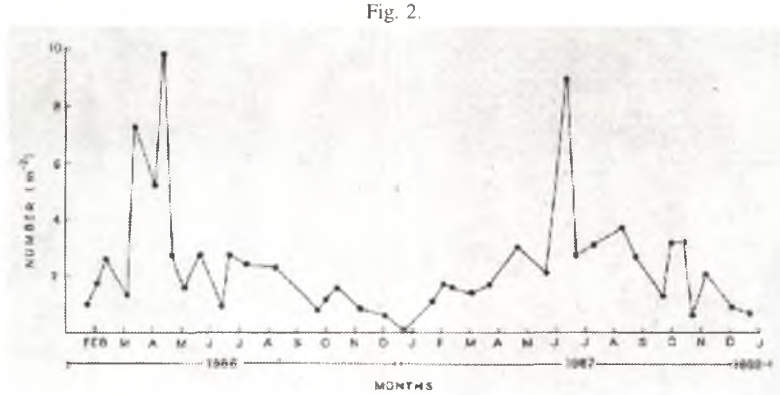

Fig. 3.

Fig. 1: Climatic data for Naukuchiatal during February 1986–January 1988; Fig. 2: Variation in live shoot and dead shoot biomass during 1986–88 (Vertical bars represent ± S.E.); Fig. 3: Variation in the density of insects during 1986–88.

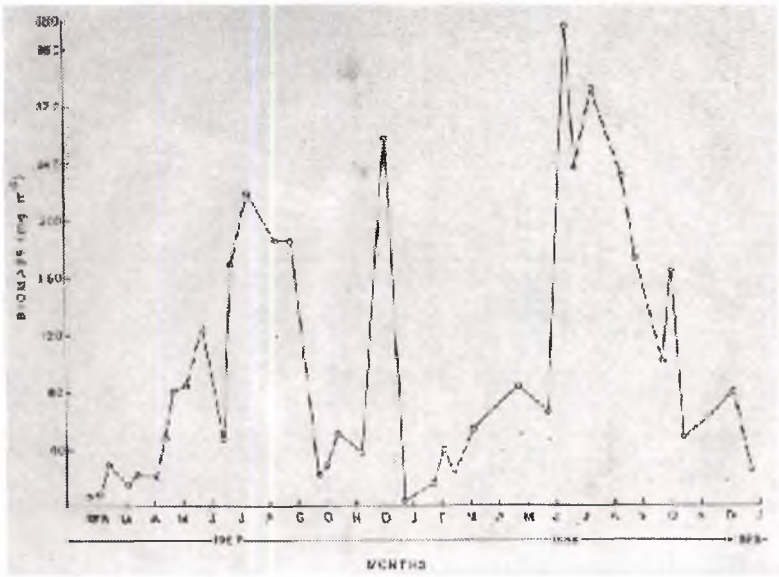

Fig. 4.

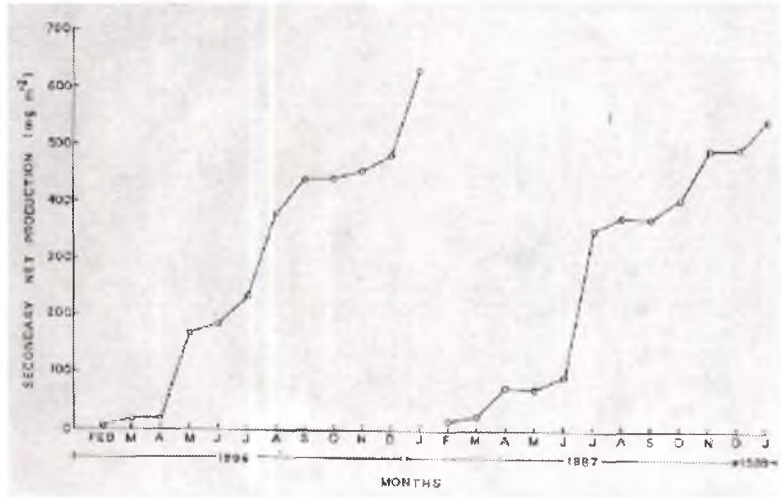

Fig. 5.

Fig. 4: Variation in the biomass of insects during 1986–88; Fig. 5: Cumulative net secondary production of herbivores during 1986–1988.

OBSERVATIONS AND DISCUSSION

Primary Producers

A total of 38 plant species were recorded. 36 species were present during second half of June to October, but far fewer were present during November to February (12) and March to first half of June (17). The biomass of live shoot was lowest in March in both the years, increased during June to September, and was at a peak in September

Table 1
Distribution of aboveground species and individuals: values in parenthesis represent percentage.

Taxonomic group	1986–87		1987–88	
	number of species	number of individuals	number of species	number of individuals
Orthoptera	12 (17.6)	115 (22.3)	12 (17.4)	211 (46.3)
Coleoptera	21 (30.9)	38 (7.3)	19 (27.7)	42 (9.2)
Heteroptera	10 (14.7)	259 (50.1)	10 (14.5)	70 (15.3)
Homoptera	2 (2.9)	3 (0.6)	2 (2.9)	9 (1.9)
Hymenoptera	8 (11.6)	39 (7.5)	9 (13.0)	65 (14.2)
Diptera	8 (11.6)	24 (4.6)	5 (7.2)	19 (4.2)
Lepidotera	3 (4.4)	6 (1.2)	7 (10.1)	15 (3.3)
Blattaria	2 (2.9)	25 (4.6)	2 (2.9)	19 (4.2)
Odonata	1 (1.5)	6 (1.2)	2 (2.9)	5 (1.2)
Dermaptera	1 (1.5)	2 (0.4)	1 (1.4)	1 (0.2)
Total	68 (100.0)	517 (100.0)	69 (100.0)	456 (100.0)

to December to 1986 and 1987 (Fig. 2). The increase in dead shoot biomass occurred 2-3 months later and was greatest in winter when annual plants had senesced (Fig. 2).

Above ground primary productivity was higher in 1987–88 (506 g m^{-2} $yr-1$) than in 1986–87 (183.9 g m^{-2} yr^{-1}).

The increase in aboveground productivity during the rainy period is attributed to the lush growth of various species with the advent of monsoon in June. The subsequent decline in live shoot biomass is due to the death and withering of annual plants and tillers of perennial grasses following maturity due to dry weather.

Secondary Producers

Species Composition and Trophic Components

A total of 88 species were collected of which 48 were recorded in both years. Table 1 lists number of species and individuals of different orders. Of the total number of individuals collected, 71.7% were herbivores, 14.9% omnivores, 8.1% predators, 5.% saprophytes, and 0.3% parasites.

Van Hook (1971), Rogers & Woodley (1976), Kaushal & Vats (1984a) Singh & Yadav (1993) have also reported that herbivores were the dominant insect group in comparison to other trophic levels in grassland communities which was observed in this study also.

Density

The data revealed that inter annual variations in population density occur: in 1986–87, population density varied from 0.1 to 9.8 m^{-2} whereas in 1987-88, population density ranged from 0.6 to 6.9 m^{-2} (Fig. 3).

Nymphs comprised of 6.9% and 16.0% of the total density in 1986-87 and 1987-88, respectively.

Biomass

The data presented in Fig. 5 reveal that variations also occur in biomass values. In 1986-87, two peaks of biomass were recorded. The first peak was recorded in rainy period when biomass increased from 6.9 to 219.6 mg m^{-2}, then declined for a short period, and the second peak (256.4 mg m^{-2}) was recorded in the winter season due to adult insects. Minimum biomass (2.9 mg m^{-2}) was recorded in the winter season. Maximum biomass in both peaks was governed by adults.

In 1987–88, biomass ranged from 16.6 to 333.6 mg m^{-2} (Fig. 4). Biomass values then declined in the winter season to minimum (24.6 mg m^{-2}) in January.

Nymphs contributed 6.2% and 4.5% in 1988–87 and 1987-88 towards total biomass, respectively.

A positive correlation was obtained between density and biomass in 1986–87 ($r = 0.799$; $p \leq 0.01$, $n = 19$) and in 1987–88 ($r = 0.853$; $p \leq 0.01$, $n = 19$).

Maximum density (9.8 m^{-2}) and biomass (333.8 mg m^{-2}) reported for the total aboveground insects in this study are lower than that reported in most other studies, 19.3 to 439.2 m^{-2}, and 16.7 to 5880.0 mg m^{-2} (Van Hook, 1971; Riegert *et al.* 1974; Kaushal & Vats, 1984a).

Maximum density obtained in summer (1988–87) and rainy period (1987–88) can be related to the availability of food (primary productivity), favourable moisture and temperature conditions. Minimum values were obtained in winter season when productivity, rainfall and temperature were low.

Gillon (1983), Kaushal & Vats (1984a), Basset & Arthington (1992) have also reported maximum density of insects during the rainy period. Unfavourable conditions i.e. extremely low and high temperatures during dry conditions result in decrease in insect population density (Gillon, 1983; Basset & Arthington, 1992).

Secondary Production

The tissue production estimates of herbivores in the present study is based on the calculations of the mean biomass of herbivores on each collection date during 1986-88. Cumulative net secondary production was 628.6 mg m^{-2} in 1986–87 (13.6 kJ m^{-2} yr^{-1}) when converted to Joules by multiplying with 22 J mg^{-1} after Kaushal & Vats, 1984b) and 545.0 mg m^{-2} yr^{-1} (12.0 kJ m^{-2} yr^{-1}) during 1987–88 (Fig. 5). The mean net secondary production was 586.8 mg m^{-2} yr^{-1} (12.9 kJ m^{-2} yr^{-1}) during 1986–1988.

Grasshoppers, particularly acridids, being the dominant group in terms of density and biomass, accounted for 65.6% and 91.4% of the net secondary production due to herbivores during 1986–87 and 1987–88, respectively. It is thus evident that contribution to net secondary production by herbivores belonging to other groups is very low compared to acridids.

Wiegert (1965) reported a secondary production of 41.84 kJ m^{-2} yr^{-1} by grasshoppers in the Michigan old–field. Van Hook (1971) reported a net production of 109.54 kJ m^{-2} yr^{-1} of grasshoppers in the Tennessee grassland. Kaushal & Vats (1984) reported a net production of 104 and 51 kJ m^{-2} yr^{-1} in a grassland at Kurukshetra. Kohler *et al.* (1967), reported that secondary production by grasshoppers in a grassland in Germany was 3407 kJ m^{-2} yr^{-1}. Low secondary production of herbivores (12.9 kJ m^{-2} yr^{-1}) in the present study could be attributed to low population density of insects.

Table 2
Mean concentrations (%) of Na, Ca, K in different trophic components (± SE).

Biological material	Sodium	Calcium	Potassium
Live shoots	0.647 + 0.01	0.099 + 0.002	0.139 + 0.002
Standing dead	0.017 + 0.003	0.033 + 0.001	0.035 + 0.002
Herbivores	0.057 + 0.008	0.085 + 0.003	0.118 + 0.002
Predators	0.076 + 0.003	0.090 + 0.001	0.098 + 0.006
Omnovores	0.049 + 0.001	0.083 + 0.002	0.102 + 0.007
Saprophytes	0.043 + 0.002	0.080 + 0.001	0.018 + 0.001

As a proportion of net primary production, secondary production was only 0.34% and 0.11% in 1988–87 and 1987–88, respectively. This value falls in the range of 0.006 to 2.1% reported in most other studies (Smalley, 1960; Odum *et al.* 1962; Wiegert, 1965; Van Hook, 1971; Rodell, 1977; Kaushal & Vats, 1984b; Köhler *et al.* 1987).

Concentration of Na, Ca and K

Vegetation

Concentration of Na, Ca and K were determined in live shoot and standing dead components of the grassland. The results are expressed on an ash–free dry–weight basis in Table 2. Expressing results on an ash–free dry–weight basis removes differences among components due to ash–composition differences and permits direct comparisons for each element independent of other elements (Reichle *et al.* 1969).

K was found in highest concentration in green vegetation. The mean concentration was 0.139%. This nutrient is generally stored as salts in vascular sap, it is lost at fairly rapid rates by leaching, and relatively low concentrations (0.035%) are found in dry material. Mean Ca concentration in green components of the grassland was 0.099%. The level of this element was low (0.038%) in standing dead material. Na, for which there appears to be no essential metabolic need in plants, was low in both green (0.647%) and dead (0.017%) vegetation.

Trophic Components

The mean concentration of Na in the predator trophic level (0.076%) was not significantly higher than the concentration in the herbivores, the primary consumers (0.057%) ($t = 0.128$, 10 d.f.), Na concentration of omnivores (0.049%) was almost equal to that of saprophyte (0.043%) trophic levels.

Mean annual whole body content of Ca did not increase significantly from the herbivores (0.085% to predator 0.090%) trophic levels ($t = 0.167$, 10 d.f.). Ca concentration in all the trophic levels was lower than in the primary producers (Table 2).

No appreciable difference ($t = 0.043$, 10 d.f.) was seen between herbivore (0.118%) and predator (0.098%) trophic levels for K. Both predators and herbivores had a lower level of K than the primary producers (0.139%). Omnivores (0.102%) had higher concentration than saprophytes (0.018%) trophic levels.

Primary consumers have nutrients 'in excess' not because they anticipate primary consumers exploitation over them and accumulate 'consciously', but because of the limitations faced by these consumers in utilizing the available resources from the producers. Reichle *et al.* (1969), Van Hook (1971) and Werner (1983) have also reported maximum concentration of elements in the primary producers. Miller (1958), Van Hook (1971), and Werner (1983) have also reported maximum concentration of Na and minimum of K in the green vegetation.

Reichle & Crossley (1965), Van Hook (1971), Cornaby (1973) and Werner (1983) have also reported highest concentration of Na at predator level. Our results agree with these studies.

The decrease in concentration of K in the present study agree with the results of Reichle *et al.* (1969), Van Hook (1971) and Werner (1983). However, Van Hook (1971) reported that in grasslands, K levels are apparently high enough in the vegetation of require no further concentration by herbivores.

Concentration of Ca in various trophic levels in the present study was similar and suggests that Ca is not as limiting as are Na and K in this insect community.

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Laboratory Colonization and Maintenance of *Toxorhynchites splendens* (Diptera: Culicidae) with a note on its Larval Preying Capacity

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Abstract: In nature, the larvae of *Toxorhynchites* mosquitoes survive by feeding on larvae of other mosquitoes whereas the adult females feed on honey or plant sap in contrast to the blood sucking habit of other female mosquitoes. *Toxorhynchites splendens* was tried to be reared in laboratory conditions under controlled temperature and humidity allowing adults to feed on honey and larvae to feed on *Culex quinquefasciatus* larvae. The period of development from egg to adult, the total length of larval and pupal period, the mean emergence rate of adults from pupae, the life span of Adults etc. were recorded. The feeding capacity of the larvae of *Toxorhynchites splendens* on prey larvae varied with different instars. The study revealed that this species can be mass reared keeping in view its suitability in different biomedical research. It can also be used as a biocontrol agent for suppression of the population of other mosquitoes.

Keywords: Colonization, *Toxorhynchites splendens*, Life history, *Culex quinquefasciatus*, Preying capacity

INTRODUCTION

Toxorhynchites species are very large sized mosquitoes. The adult females of this mosquito do not take blood meal whereas the larvae are carnivorous feeding on eggs and larvae of other mosquitoes. In nature, females of *Toxorhynchites* lay eggs inside tree holes, bamboo stumps, banana leaf axils etc. where they prey upon and devour the larvae of other mosquitoes living in the same situations. In a bamboo stump or a tree hole, the existence of more than one or two larvae of *Toxorhynchites* sp. is very rare. It is due to the effect of cannibalism within the species in the absence of the larvae of other species and finally the stronger one survives. The adults feed on honey or plant sap.

Wild caught *Toxorhynchites splendens*, were tried for rearing in the laboratory conditions for its large scale production for utilization in arbovirus studies and to study further its efficacy as a biological control agent for immatures of container breeding mosquito species. This paper reports on colonization and maintenance of *T. splendens*

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along with the observation on life–cycle and preying capacity of larval stages of this species during development in laboratory conditions.

MATERIALS AND METHODS

Toxorhynchites larvae for the initiation of colony were collected from bamboo stumps in a bamboo growing area near Dibrugarh, Assam. Generally one or occasionally two larvae of *Tx. splendens* were found in one bamboo stump. Larvae were kept individually in plastic vials (3 cm diameter and 4 cm long) and were allowed to feed on the laboratory reared larvae of *C. quinquefasciatus*. After attaining the pupal stages, they were transferred into a plastic bowl (17.5 cm diameter) placed inside a cage (L 70.5 cm × B 52.5 cm × H 35 cm) made up of wooden frame and wire mesh for emergence. After emergence, the adults of *Tx. splendens* were offered honey, soaked in a cotton pad which was suspended from the roof of the cage. Plastic bowls with diameter of 17.5 cm and depth of 7 cm were used as ovitraps. A piece of black cloth was used for wrapping the inner wall of the bowl where water was kept filled upto $\frac{1}{3}$ rd of the bowl.

After oviposition, the ovitraps were removed from the adult cage and the eggs were allowed to hatch. The larvae, thus hatched, were kept separately in plastic vials (size 3 cm diameter & 5 cm long) and allowed to feed on the Ist instar larvae of *C. quinquefasciatus*. When they grew into the 2nd instar, they were given the 2nd and 3rd instar larvae of *C. quinquefasciatus* and the larval development period was recorded. Similarly, the other stages of *Tx. splendens* were also given the known number of larvae of *Cx. quinquefasciatus* as prey to determine the feeding capacity of different instars, rate of feeding etc. The development period of each instar, pupal period, adult's life–span (male & female), adult's and larval feeding habit, etc. were recorded. The laboratory was maintained at 26 ± 2° C temperature and relative humidity of 70-80%.

RESULTS AND DISCUSSION

Life History

Males and females of *Tx. splendens* adults were released into the cages and allowed to feed upon honey. Females took 9.1 (±0.8) days for laying first batch of egg. It is found that one female lays about 200–230 eggs in her lifetime and egg laying starts from 10–12th day after emergence from pupa and continues for 19–21 days in laboratory conditions. Eggs were oval in shape, white in colour and later turned into greyish. Egg surface was observed to be spinose under light microscope. Observations on eggs showed that a minimum time of 24 hours was required for hatching and maximum hatching (about 60%) was achieved between 30–48 hours. While rearing *Tx. moctezuma* in Trinidad, Tikasingh and Martinez (1992) reported that a maximum of 42 hrs. was required for hatching the eggs. The egg hatching time may be different with the fluctuations of temperature.

When the egg hatching completed, the larvae were kept separately in plastic vials allowing to feed on first instar larvae of *Cx. quinquefasciatus*. It was observed that under the ambient laboratory conditions as mentioned above, the first instar larvae took 3.6(±0.6) days to grow into the second instar. Similarly, the periods for development from 2nd to 3rd instar 4.5(±0.5) days, and 3rd to 4th instar 5.4(±0.5) days were

Table 1
Biology of *Toxorhynchites splendens* studied in the laboratory

Variables	No. of observation	Span (days)	
		Range	Mean ± SD
Oviposition	30	8–10	9.1 ± 0.8
Egg hatching*	20	1.25–2.0	1.5 ± 0.5
1st to 2nd instar	30	3–5	3.6 ± 0.6
2nd to 3rd instar	30	4–5	4.5 ± 0.5
3rd to 4th instar	30	5–6	5.4 ± 0.5
4th instar to Pupa	30	4–6	5.4 ± 0.6
Pupa to Adult	30	4–5	4.5 ± 0.5
Life–span (male)	30	16–19	17.8 ± 4.2
Life–span (female)	30	25–30	28.6 ± 5.4

*Each observation with 50 No. of eggs.

recorded. The 4th instar larvae took 5.4(±0.6) days for attaining pupal stage showing a total larval period of 18.8(±3.2) days. The pupal stage lasted 4.5(±0.5) days for adult emergence. The mean emergence rate of adults from pupae was found to be 90%. It was calculated that *Tx. splendens* took 22–29 days for development from egg to adult under the maintained temperature (26 ± 2° C) and humidity (70–80%) in the laboratory. The life span of adult of *Tx. splendens* mosquitoes was recorded to be 17.8± (±4.2) days in case of male and 28.6(±5.4) days in case of female mosquito.

Larval Feeding Capacity

In general, the larvae of *Toxorhynchites* are carnivorous. During rearing of *Tx. splendens* larvae individually in the laboratory preying upon the larvae of *Cx. quinquefasciatus*, observations were made to determine the feeding capacity of different instars and rate of feeding (per day) etc. Table 2 shows the number of larvae of *Cx. quinquefasciatus* consumed by the different instars of *Tx. splendens* and feeding rate per day of different instars of *Tx. splendens*. The results based on 30 *Tx. splendens* reared from egg to adult in the laboratory revealed that 1st, 2nd, 3rd and 4th instars consumed the number of prey larvae on an average 61.7(±6.4), 47.6(±2.3), 58.0(±4.2) and 60.1(±2.0) respectively in their developmental period. It was also seen that the 1st, 2nd, 3rd and 4th instar consumed the number of prey larvae on an average 14.6(±1.3), 10.3(±0.8), 11.8(±0.9) and 13.8(±3.4) per day respectively. First instar larvae *Tx. splendens* were unable to consume the 3rd and 4th instars of prey larvae although they were observed to attack and knock them down.

From our observations, it is clear that the *Tx. splendens* larvae consume a considerable number of prey larvae during their developmental period. A single *Tx. splendens* larva can consume on an average 227.5(±5.2) prey mosquito larvae of different instars during its developmental period. In case of *Tx. moctezuma*, the consumption of number of prey larvae was recorded to be in the range of 258–269 (Tikasingh and Martinez, 1992). The consumption rate of *Tx. moctezuma* was high in comparison to

Table 2
Observation on prey (*Cx. quinquefasciatus*) larvae consumption by *Toxorhynchites splendens* reared from eggs to adult in the laboratory

Different instars	Prey consumption (mean ± SD)	
	Total	Per day
1st instar	61.7 ± 6.4	14.6 ± 1.3
2nd instar	47.6 ± 2.3	10.3 ± 0.8
3rd instar	58.0 ± 4.2	11.8 ± 0.9
4th instar	60.1 ± 2.0	13.8 ± 3.4

SD ± (Mean of 30 observations)

Tx. splendens which was perhaps due to the longer 3rd and 4th instar development period of the former. These studies revealed that *Toxorhynchites* mosquito is a good predator preying upon the other species of mosquitoes and therefore, it can be used as potential control agent for suppression of the population of other mosquitoes. The use of *Toxorhynchites* mosquitoes as control agents was first proposed by Colledge (1911). There are few reports of using *Toxorhynchites* as biocontrol agent for suppression of *Aedes aegypti* mosquitoes, the vector of Dengue haemorrhagic fever (Panicker and Geetha Bai, 1983; Chadee, 1985; Gerberg, 1985).

Our study further reveals that *Tx. splendens* can be reared successfully in laboratory conditions for mass production so as to utilize in different biomedical research. As the larvae and adults of this mosquito are very large in size and support the growth of some of the arthropod borne viruses, it can be used as an experimental model for arbovirus studies. Moreover, as the adult females do not take bloodmeal, any accidental escape of these during arbovirus study from the insectary will not cause any problem.

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Seasonal Patterns of Root Borer, *Emmalocera depressella* Swinhoe on Sugarcane in India

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Abstract: Studies on the seasonal patterns of sugarcane root borer, *Emmalocera depressella* Swinhoe in early cultivar 'Co 89003' were conducted during 1992-'93. Results indicate that the root borer incidence was significantly -vely correlated (r=-0.531) with maximum temperature and +vely correlated (r=0.580) with relative humidity. The seasonal index worked out based on time series showed that the maximum temperature ranging from 31° to 34° C and relative humidity (RH) from 48.2 to 78.4% during July - August were congenial for multiplication of root borer which was observed at peak during Aug-Sep-Oct. months. Population of root borer remained low during May-June months.

Keywords: Root borer, weather parameters, seasonal index.

INTRODUCTION

Sugarcane is an important commercial crop of India. The root borer, *Emmalocera depressella* Swinhoe is one of the most destructive pests in sub-tropical cane growing belts causing about 10 per cent reduction in yield (Gupta and Avasthy, 1952). Knowledge on the seasonal patterns of root borer is a prerequisite for development of a sound pest management programme. To this end, a study on the seasonal variation of sugarcane root borer was conducted and results are presented in this paper.

MATERIAL AND METHODS

Field experiment was laid out at the farm of Sugarcane Breeding Institute, Regional Center, Karnal with an early sugarcane cultivar 'Co 89003' planted in an area of 0.25 acre during 1992-'93. All standard agronomical practices were followed but without insecticidal application. The field was divided into 10 uniform quadrats. In each quadrat 30 tillers were taken at random for observation commencing from 15th May upto 30th June (there was no clump formation till 30th June) while 5 clumps were taken at random from 15th July and repeated at fortnightly intervals. There were total 24 observations till April end (in a Year). The average per cent incidence recorded for each observation during different fortnights was correlated with various weather

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Table 1
Correlation among root borer incidence, its growth and weather parameters.

Parameter	Correlation coefficients	
	Root borer incidence	Growth of root borer
Maximum temperature	-0.531*	0.269
Minimum temperature	-0.331	0.465
Relative humidity	0.580*	0.370
Rainfall	-0.365	-0.034
Sunshine	-0.472	-0.454
Wind speed	-0.461	-0.248

* Significant at 1 per cent level.

factors viz; average maximum and minimum temperature, relative humidity, sunshine, wind speed and total rainfall over the preceding fortnight of the observation and correlation coefficients were worked out.

The growth of root borer population was estimated by obtaining the difference between per cent incidence of root borer recorded during a particular fortnight and that recorded during the preceding fortnight. This growth value was used to work out correlation with the weather parameters mentioned above. Regression equation using least square method was worked out in order to arrive at prediction of root borer incidence at different crop growth stages.

Another important aspect of population variation is the temporal variation (in relation to time). To analyse differences in time to time variation, semi-variogram was calculated using Campbell (1978) method.

Analysis of time series was employed to determine the seasonal variations of root brer population. Time series using multiplicative models are widely adopted for analysis of data which varies with time (Croxton *et al.* 1969). It is hypothesized as a product of trend (T), cyclical fluctuation. Average fortnightly incidence was divided by the moving average to obtain an estimate of S, the seasonal variation. To remove errors that accrue due to irregular trends the entire data was once again adjusted by multiplying the index of values with 100 which served as correction factor (Croxton *et al.* 1969).

Climatological data for the above period was recorded at the observatory of C.S.S.R.I., Karnal.

RESULTS AND DISCUSSION

Correlation coefficients among weather elements and root borer population and its growth rate are presented in Table 1.

Maximum temperature was found to have a significant -ve correlation (r=-0.531) while relative humidity was significantly +vely correlated (r=0.580) with root borer incidence. However it was also observed that population growth of root borer was not

influenced by any of the above mentioned climatic factors. This shows that population growth is independent of weather factors but is probably related to the fecundity of females in generation 't' or to the survival of their progeny in generation 't+1' or to both processes. The partial regression coefficient worked out individually between per cent incidence and maximum temperature and relative humidity also showed that maximum temperature was -vely correlated and relative humidity was +vely correlated with the build up of root borer. The regression equation between the per cent incidence (Y) and the maximum temperature (X_1) worked was

$$\begin{aligned} Y &= 64.529 - 1.20060X_1 \\ &\quad\ (-2.9459) \\ &\quad\ (0.316) \end{aligned} \tag{1}$$

This function had a coefficient of determination of 28 per cent.

Regression equation with relative humidity (X_2) was

$$\begin{aligned} Y &= 6.01572 + 0.47016X_2 \\ &\quad\ (3.3616) \\ &\quad\ (0.189) \end{aligned} \tag{2}$$

This had a coefficient of determination of 34 per cent.

The R^2 values for maximum temperature and relative humidity showed that these two weather factors gave a less account of variability. However, together these two factors gave a higher account of variability upto 44 per cent and the equation worked out was:

$$\begin{aligned} Y = 35.3783 &- 0.8058X_1 + 0.3572X_2 \\ &(-2.0468) \quad (2.5082) \\ &(0.393) \quad\ (0.142) \end{aligned} \tag{3}$$

Multiple linear regression equation obtained for all the climatic factors as independent variables was:

$$\begin{aligned} Y = &-29.364 + 2.419X_1 - 2.748X_2 + 0.886X_3 + 0.120X_4 \\ &\quad\ (1.582) \quad (2.218) \quad\ (3.285) \quad\ (1.317) \\ &\quad\ (1.574) \quad (1.239) \quad\ (0.698) \quad\ (0.913) \\ &0.003X_5 - 4.426X_6 \\ &(0.001) \quad\ (-4.530) \\ &(1.777) \quad\ (0.976) \end{aligned} \tag{4}$$

The coefficient of multiple determination (R^2) was 0.83. thus, R^2 value gave a highly significant account of variability and this indicated that variability in incidence

could be explained to the extent of 83 per cent due to combined effect of these climatic factors.

In order to arrive at prediction of root borer incidence at different crop growth stages, the relationship between pest incidence and RH and maximum temperature was worked out for a model given below:

$$Y = a + \alpha\,(\mathrm{NDAP})\alpha + \beta\,(\mathrm{MT}) + \gamma\,(\mathrm{RH}) + e$$

Where, Y = estimate of pest incidence at different stages of growth

a = intercept value

α, β, γ = partial regression coefficients corresponding to NDAP (number of days after planting), maximum temperature and relative humidity

e = error that follows assumptions underlying least square method in fitting regression equation

The prediction equation for estimating root borer incidence at various stages is given below:

$$\begin{array}{lccc} Y = 30.1998 + 0.00705 & (NDAP) - 0.7138(MT) + & 0.3725(RH) & (5) \\ \quad (0.031) & (0.573) & (0.160)\cdots SEvalue & \\ \quad (0.225) & (-1.244) & (2.315)\cdots\text{`}t\text{'}value & \end{array}$$

Values for NDAP and mean values for MT and RH over the preceding fortnight of observation should be substituted in the above equation. The coefficient of multiple determination (R^2) was 0.44. Fairly high values gave a high account of variability.

The semi - variogram obtained indicated that there was a possibility of temporal association for root borer population upto 5 fortnights, (Fig. 2).

The seasonal index worked out based on time series has been depicted in Fig. 1. Root borer incidence had a non-linear trend throughout the period of study. Peak incidence was observed during end August to end of October when maximum temperature ranged from 31° C to 34° C and RH from 48.2 to 78.4 per cent. Though the infestation of root borer starts in May itself, the population remains very low during pre-monsoon period. The critical scrutiny of incidence and weather factors revealed that during May-June the high temperature and low humidity conditions were not suitable for the pest incidence as shown in figure 1. However, during July-August when maximum temperature fluctuated between 31° C - 34° C and relative humidity between 40 - 75% the population started multiplying and a rising trend was seen in the July - August itself. As there is overlapping of generations and with a life cycle of 45-55 days the cumulative peak incidence of root borer in the field is reflected in the month of Sept. and October. The cold season from November to February seems to be unfavourable for this insect when its reproductive and growth rate might have been affected by the

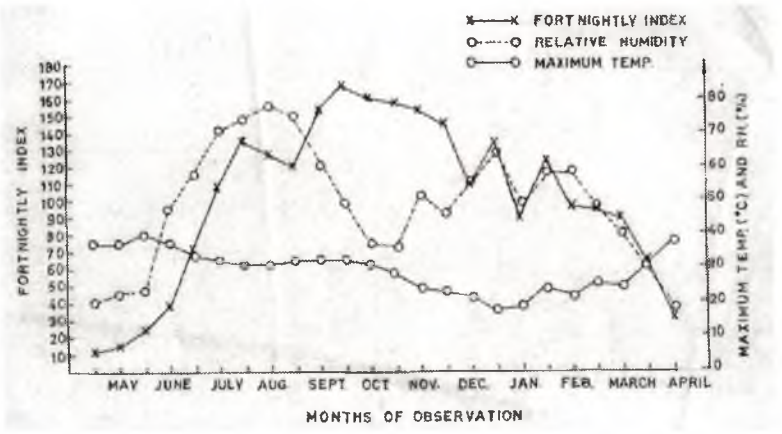

Fig. 1 Seasonal index of root borer, *Emmalocera depressella* Swinhoe

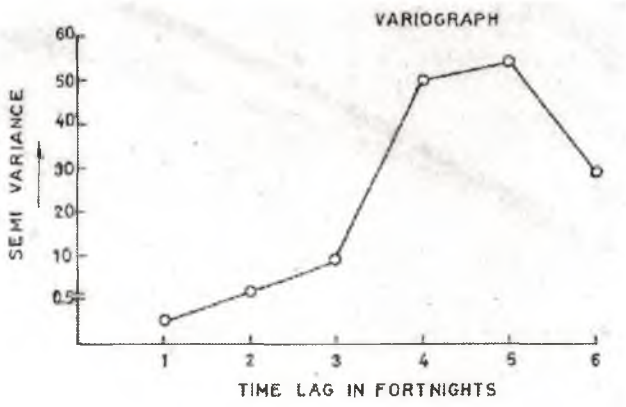

Fig. 2 Semi-variogram for temporal relation

low temperature, as a result of which it undergoes diapause. However, during this diapause there might be a slight natural mortality and with the onset of summer during March-April, population further declines. Thus, the time lag for the next population rise might be about 6 months (Fig. 2). The temperature ranging from 31° C to 34° C and high humidity could be regarded as critical factors for its maximum development, while very low and very high temperature with very low humidity levels were unfavourable for the pest. Gupta (1953) also reported peak incidence of root borer in August - September when temperature and humidity conditions were very congenial.

E. depressella does not appear to have natural enemies of much consequence. Current methods of control of this pest involve mass trapping of moths by using of light traps and by applying chemicals. The present study has shown the pest to be active throughout the year with peak incidence between August to November. This information will be of use in directing appropriate pest control measures during the right season.

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Antifeedant property of *Lantana camara var. aculeata* and *Aloe vera* leaves against teak skeletonizer, *Eutectona machaeralis* Walk. (Lepidoptera: Pyralidae)

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Abstract: Methanolic leaf extracts of two plants *Aloe vera* and *Lantana camara var. aculeata* have been screened for antifeedant property against teak skeletonizer, *Eutectona machaeralis*, at various concentrations. Extracts of both plants were equally effective in reducing the food consumption rate. Treated leaves with *L. camara* extract at 2.0 per cent and above provided 80.21 and 87.35 per cent leaf protection respectively. Larval mortality due to starvation was observed at 3.0 per cent and above which can be considered as a threshold concentration for absolute activity. Effective concentration for 50 per cent protection (EC_{50}) was also calculated.

Keywords: antifeedant, *Aloe vera, Lantana camara, Eutectona machaeralis*, EC_{50}, plant extracts.

INTRODUCTION

Teak (*Tetctona grandis*) which is one of the valued timber tree species, has been studied extensively for the insect pest fauna. Nearly 288 insects have been found associated with teak all over the country (Tewari, 1992). Teak skeletonizer, *Eutectona machaeralis* is one of the serious pests causing defoliation and premature shedding of the leaves, which ultimately affects the growth (Beeson, 1941). The biology and distribution of this species has been studied by Beeson (1941) in detail. Later, Singh & Gupta (1978) tested the relative toxicity of 20 insecticides against its third instar larvae. Except this, not much studies have been made on the management of this pest.

The environmental hazards posed by synthetic pesticides provide an impetus for the investigations into some ecofriendly and biorational alternatives. The higher plants were identified as the richest source for renewable bioactive chemical with multifacial effects on insects (Arnason *et al.*, 1989). Anti-insect chemicals from plants may be variously toxic, repellent, cause sterility, modify behaviour or reduce feeding (Feeny,

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1983; Bowers, 1985; Morgan & Mandava, 1987; Saxena & Tikku, 1990; Singh, 1993). Any one of these properties may be useful to regulate the pest population. A most notable recent success in this direction is the commercial development of neem, which was used in India and elsewhere for various effects (Pradhan *et al.* 1962; Isman *et al.* 1991; Singh, 1993 a,b). Most of the above evaluations have been restricted to the insect pests of agricultural importance, except for a few reports on forestry insect species (Bhandari *et al.* 1988; Meshram *et al.* 1994; Kulkarni *et al.* 1996). In the present study the antifeedant property of the leaf extracts of two plant species viz., *Aloe vera* (medicinal plant) and *L. camara var. aculeata* were tested against the larvae of teak skeletonizer, *E. machaeralis*.

MATERIALS AND METHODS

The larvae of *Eutectona machaeralis* were collected from adjoining teak plantations and reared in the laboratory on tender leaves. Leaves of *A. vera* and *L. camara* were shade dried in the laboratory, powdered and subjected to extraction with the help of "soxhlet apparatus", using methanol as solvent. The dried residue was redissolved in methanol to get 5.0 per cent stock solution which was further diluted in distilled water as desired (Table 1). A few drops of Triton AE were added in each dilution as an emulsifier. Each concentration was then sprayed on both the surfaces of leaves by using a hand automizer and allowed to dry at room temperature. Untreated leaves were exposed in control. Each treatment was replicated five time. Larvae starved for 24 hours were used for the tests. A single starved larva was exposed to treated and untreated leaves in a covered petri dish for 24 hours, after which leaf area consumption was measured with the help of a leaf area meter "systronics 211". The per cent protection or antifeedant activity was calculated by the method of Gujar & Mehrotra (1988). The effective concentration for 50 per cent protection (EC_{50}) was calculated by subjecting the data to probit analysis (Finney, 1971). The experiments were carried out at temperature 30+2 C, R.H. 60 + 5% and photoperiod of 12h L: 12h D, as pertained in nature.

RESULTS AND DISCUSSION

Antifeedant activity was exhibited by both the extracts tested, evident by reduced food consumption compared to untreated control. A negative correlation was shown by larvae between increased concentration of the extracts and reduced rate of feeding, corresponding to increased leaf area protection (antifeedant activity over control) (Table 1). The least concentration for the antifeedant activity was 0.5%, below which no activity was obtained. Activity increased to its threshold at the concentration 2.0 per cent and above, at which feeding was reduced to 8.35 per cent ($P<0.01$) and 6.30 per cent ($P<0.01$) against 34.90 per cent in control corresponding, respectively to the leaf area protection of 73.78 per cent ($P<0.05$) and 80.21 per cent ($P<0.05$) against nil in untreated control. Further increase in the concentration resulted in mortality in some larvae due to starvation. This can be taken as an absolute concentration against *E. machaeralis* in laboratory. The effective concentration for 50 per cent protection (EC_{50}) in treatments against control was worked out to be 1.046 per cent with upper and lower fiducial limits of 0.476 and 2.300 respectively in case of *A. vera* and 1.025

Table 1
Leaf area consumption by the larvae of *E. Machaeralis* and leaf protection over feeding in control.

Treatments (%)	Leaf area consumption* (in % /24 hr/larvae)	Leaf protection over feeding in control (%)'
A. vera leaves 0.5	23.10 (28.66)	27.46 (30.96)#
A. vera leaves 1.0	16.10 (23.28)	49.44 (44.06)
A. vera leaves 2.0	8.39 (16.61)	73.78 (59.43)
A. vera leaves 3.0	7.60 (12.42)	76.13 (66.60)
L. camara leaves 0.5	22.69 (28.37)	28.73 (31.86)
L. camara leaves 1.0	20.66 (26.91)	35.39 (33.33)
L. camara leaves 2.0	6.30 (14.48)	80.21 (63.64)
L. camara leaves 3.0	4.08 (7.04)	87.35 (76.76)
Solvent treated (control)	28.80 (32.19)	–
Untreated (control)	34.90 (36.19)	–
SE	3.80	8.22
CD 5%	7.721	16.705
1%	10.361	22.416

* - Based on total leaf area provided.
# - Figures in parentheses are arc sin transformed values.

Table 2
Effective Concentration for 50 per cent leaf protection (EC_{50}) against *E. Machaeralis* larvae.

Plant extracts	Heterogeneity	EC_{50}	Fiducial limits
A. vera leaves	$X^2_{(2)} = 0.08383$	1.04691	0.47639 to 2.300701
L. camara leaves	$X^2_{(2)} = 0.52707$	1.02507	0.55528 to 1.89233

per cent with upper and lower fiducial limits 0.555 and 1.892 per cent in case of *L. camara* extract in laboratory (Table 2).

Among the natural products of botanical origin tested so far, neem is the most versatile antifeedant along with many other behavioural and physiological effects (Schmutterer, 1995). Kulkarni *et al.* (1996 a) have tested bioactivity of neem against the 5th instar larvae of *E. machaeralis*. They found that methanolic neem seed extract (MNSE) was very effective as antifeedant at 2.0 and 1.0 per cent concentrations with 75.44 and 70.15 per cent leaf area protection, respectively. Feeding MNSE treated leaves to 5th instar larvae, affected growth of the larvae and caused larval, pupal mortality.

Preliminary screening of antifeedant activity of several plant species including 0.5 per cent leaf extract of *A. vera* was carried out against *E. machaeralis* by Meshram *et al.* (1994). They found that leaf extract of *A. vera* was very effective as antifeedant.

The earliest report on the deterrence property of *L. camara* is against *H. machaeralis* (E. machaeralis) by Chatterjee & Sebastian (1965). They also suggested application of *L. camara* as a future insecticide. Ironically, no further work followed till date on this aspect. Methanolic leaf extracts of 6 plant species including *L. camara var. aculeata* and *A. vera* were tested against the larvae of popular defoliator, *Colstera cupreata*, bamboo leaf roller, *Crypsiptya coclesalis* and mahaneem webworm, *Atteva fabriciella* by Kulkarni *et al.* (1996b). Although, both the extracts reduced feeding but leaf extract of *L. camara* was found to be more effective as compared to *A. vera*. Leaf extract of *L. camara* has also been tested against *Henosepilachana vigintioctopuntata* (Chitra *et al.* 1990).

The present activity exhibited by *A. vera* and *L. camara* may be due to the active toxic groups like Aloin (Anonymous, 1985) and Lantadane A (Anonymous, 1962), present in the leaves. The possibility of some other chemical group responsible cannot be ruled out and has to be elucidated in future along with some other bioactivities.

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On a New Species of *Friona* Cameron (Hymenoptera: Ichneumonidae) from India

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Abstract: A new species of *Friona* Cameron (1902) Collected from India (Maharashtra: Aurangabad) is described and illustrated.

Keywords: *Friona aurangabadensis*, sp. nov. from India.

INTRODUCTION

The Oriental region restricted genus *Friona* Cameron (1902) described on type-species *F. striolata* belongs to the tribe Gorphini of the subfamily Mesosteninae (Gupta 1987). Townes (1970) provided generic diagnosis and key to distinguish it. Townes, Townes & Gupta (1961) Synonymised *Lactolus albomaculatus* Viereck (1914) under genus *Friona*. Extensive works of *Friona* were attempted by Brulle (1946), Smith (1958-'60), Tosquinet (1903), Szepligeti (1908-1916), Morley (1914), Dutt (1923), Uchida (1930-'32) and Betrem (1941).

Townes et. al. (1961) and Gupta (1987) catalogued 29 species of *Friona* from the Indo-Australian Region. Only seven species of *Friona* have been recorded in the diversified ecological niches in India. A reliable key for the Indian species of *Friona* has been provided for the first time.

Friona aurangabadensis. sp. nov. (Figs 1-5)

Male:

Body length 9.5 - 10.0 mm (Fig.1) Head (Fig.2) in profile 1.90 x as long as deep; when viewed from front 0.90 as long as broad; vertex finely punctulate; ocellar-triangle coarsely punctate; inter-ocellar distance same as their diameter; ocello-ocullar distance same as their diameter; ocello-ocular distance 1.25 x the inter-ocellar distance; front with a median carina laterally with 3-4 irregular wrinkles at the base of antennal scrobes, concave, weakly punctate; antenna slightly shorter than the length of body, attenuate; 2 + 32 segmented; scape 1.1 x as long as broad; pedicel as long as broad; first flagellar segment 1.4 x as long as the second flagellar segment; terminal segment three times as long as broad; 1.8 x as long as the penultimate segment; face as long as broad,

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pubescent, punctate; clypeus 0.5 times as long as broad, distinctly separated from face, moderately convex, weakly punctate, its apical margin weakly convex; malar space as long as the basal width of mandible densely granular; mandible 1.6 x as long as broad, teeth unequal; occiput shiny, sparsely punctate, sparsely pubescent; occipital carina strong, medio dorsally acutely arched, complete, joining the hypostomal carina at the base of mandible.

Thorax twice as long as broad; collar subshiny with sparse weak punctures; pronotum glabrous, weakly punctate; epomia strong, reaching dorsal of pronotum; mesoscutum finely punctate; notolus strongly impressed, running behind the middle; scutellum at base canaliculate provided with three vertical carinae; its baso-lateral carina distinct, 2.0 x as long as broad, shagreen, trapezoid shaped; post-scutellum oval; propodeum (Fig. 3) above 1.2 x as long as broad, basal carina strong, sharp, posteriorly a pair of small crest present, its microsculpture reticulo-punctate and laterally trans-striate; pleural carina indistinct; propodeal spiracle elongate, 1.8 x as long as wide; propleurum medially roughly wrinkled, rest weakly punctate; mesopleurum below subtegular ridge strongly trans-striate, otherwise reticulo-punctate; speculum shiny; prepectal carina sinuate, sharp, anteriorly reaching to the hind corner of pronotum; postpectal carina complete; sternaulus strong, extending upto mid of mid coxa; metapleurum striagose or strongly rugose; fore and mid-trochanters 3.0 x as long as length of the trochanter-trochontellus combined, hind coxae and trochanters without long black hairs; hind femur 0.75-0.80 times as long as hind tibia; basitarsus 0.70 times as long as the length of remaining tarsal segments; tibial spurs unequal, the longer spur 0.5 the length of basitarsus.

Forewing 3.5 x as long as broad; basal abscissa of radius 0.65-0.70 the length of its apical abscissa; areolet large, pentagonal, 1.3-1.4 x as broad as high; nervulus based to basal vein by 0.25 its length; nervellar index 0.65; second recurrent vertical, broadly fenestrated; 3.0 x as long as intercubitus; second intercubitus medially transparent; basal abscissa of subdiscoideus 1.80 - 1.85 x as long as its apical abscissal second discoidal cell 2.85 x as long as broad; discocubital cell 3.11 x as long as broad. Hind wing 4.0 x as long as broad; nervellus intercepted 0.25 - 0.30 below the centre; basal abscissa of radiella 0.30-0.35 times the length of its apical abscissa; mediella moderately arched; basal abscissa of cubitella 1.65 x the length of its apical abscissa; auxillus not reaching to its margin; hamuli 1 + 8.

Abdomen 1.5 x the length of head and thorax combined; first tergite nearly as long as the second tergite; its base provided with a triangular tooth and ventrolateral carina; anteriorly finely punctate, apically subshiny, with sparse punctures; second tergite 1.5 x as long as broad, densely punctate; rest of the tergites mat to punctate; thyridium longer than wide separated from base by 2.2 x its length.

Black:

Yellowish-whitish markings on : 10 - 16 antennal segments, laterads of vertex, complete face except short median mark, clypeus except laterads and apical margin; mandibles except teeth, spot on gena palpi, transverse band on collar, posterio-lateral small mark on mesoscutum, scutellum, post-scutellum, tegula, subtegular ridge, triangular mark on baso-lateral furrow of propodeum, small spot on basal area and broad nail-like mark on propodeum, basal and apical transverse bands on first tergite and only trans-

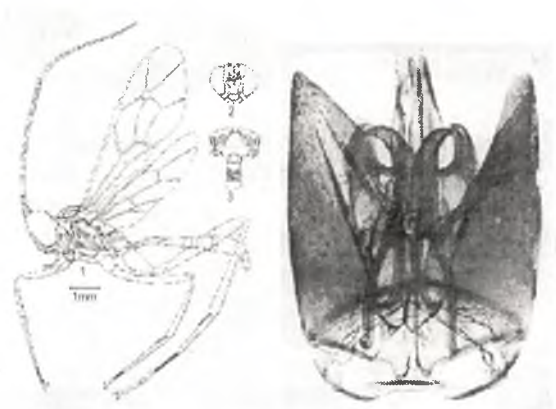

Fig. 1–3 Fig. 4 Fig. 5

Fig. 1-3. *Friona aurangabadensis* Sp. nov. Male: 1. Lateral view, entire; 2. Head front view; 3. Propodeum and first abdominal tergite dorsal view.
Fig. 4-5. *Friona aurangabadensis* Sp. nov. Male: 4. Genitalia complete; 5. Sub-genital plate.

verse apical band on rest of the tergites, 2-4 hind tarsal segments black. Fore and mid legs rufus.

Male genitalia:

(Fig. 4) Paramere gonosquamma conical, very sparsely pubescent; volsella apically blunt, with 13 bristles, aedeagus spindle shaped, ergot broad basally; sub-genital plate (Fig. 5) covered with scales, spiculum thick and short; gonoring with wide lumen.

Female:

Unknown

Holotype: Male, India: Maharastra: Aurangabad; ix. 1982 and paratypes 2 males, 19, viii. 1983, Nasik, Malaise trap coll. 2 males, Aurangabad: Bhavsingpura; 10 males, 13. xi. 1981, L. J. Kanhekar coll., 4 males, 22. xii. 1982 Himayat bag, Aurangabad (Deposited in Zoology Department, Dr. B. A. Marathwada University, Aurangabad).

Discussion

In accordance to the key appended for the Indian species of *Friona* Cameron, *F. aurangabadensis*, sp. nov. shows close resemblance with *F. rufipes* Cameron in the characters of frons, face, legs, areolet etc. However, it differs from the same in having: (i) Clypeus apically and laterally black, (ii) scutellum trapezoidal, yellow, (iii) basal area with inverted yellow triangular mark, (iv) propodeum medially reticulo-punctuate and laterally trans-striate,(v) occiput black and sparsely pubescent, (vi) mesonotum finely punctate; (vii) hind tibia apically black, (viii) propodeal apophysis distinct, (ix) pleurae immaculate.

Key to Indian species of *Friona* CAMERON

1. Anterior four legs fulvous or yellowish 2
   Anterior four legs rufus or reddish 6
2. Propodeum with 'A' mark; stigma black, with a white spot at base; face rugosely-punctate; pro and meso- thorax smooth and shiny. Bihar, Maharashtra *Octobaltea* ..... (Cameron, 1907)
   Propodeum with a broad longitudinal or transverse mark; stigma black, without white spot at the base; face punctate; pro and mesothorax punctate to rugulosopunctuate, pubescent 3
3. Propodeum with a sharp and distinct basal carina, rugosely-reticulate, aerola slightly indicated at the base; with triangular mark on the metapleurum. West Bengal *lineatipes* ..... (Cameron, 1907)
   Propodeum with a obsolete basal carina, transversely striated; areola absent; without a mark on metapleurum 4
4. Keels or striae on frons longitudinal; pleurae strongly, longitudinally striate. Meghalaya, Assam *frontella* ..... (Cameron 1904)
   Keels of striae on frons curved or transverse; pleurae weakly sparsely striate .. 5
5. Head and thorax yellow; antenna longer than the length of body. Meghalaya *curvicarinata* ..... (Cameron 1904)
   Head and thorax entirely red or brown; antenna subequal to body length. A mere color variety of *F. curvicarinata* Sikkim, Assam. *rufescens* ..... (Morely 1914)
6. Clypeus throughout black except two yellowish dots; antennal scrobes with two yellow dots; face centrally carinated. Uttar Pradesh, West Bengal, Assam, Kerala *didymata* ..... (Morley 1914)
   Clypeus laterally and apically black; face punctate, laterally and medially weakly striated; Propodeum with a broad inverted 'T' shaped yellowish mark 7
7. Propodeum with crests or apophysis, face with a triangular black, mark; propodeum, hind coxae and trochanters provided with long black hairs. Bihar *rufipes* (Cameron 1905)
   Propodeum with a pair of horn like crest or apophysis; face without a black triangular mark; propodeum, hind coxae and trochanters without long black hairs, Maharashtra *aurangabadensis* ..... sp. nov.

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Two new species and revised key to Indian species of *Dolerus* panzer (Hymenoptera: Symphyta: Tenthredinidae)

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Abstract: Two new species are described and illustrated under genus *Dolerus* panzer, which are *D. cingulatus* and *D. nigripleuris* recorded from Nagaland and Jammu & Kashmir respectively. A revised key to all Indian species of this genus is provided.

Keywords: New species, *Dolerus* panzer, Hymenoptera, Tenthredinidae, India.

INTRODUCTION

The work on Indian Dolerinae was initiated by Cameron (1876) by describing a species *Dolerus rufocinctus*. Then, after a wide gap of a century or so Muche (1983) described second species under this subfamily i.e. *Loderus mira* from India. Following this work Saini & Singh (1987) added four more species to genus *Loderus* from India. But they (1989) while describing a new species i.e. *D. tangmargensis*, and following Goulet (1986) shifted all five species earlier described under genus *Loderus* konow to *Dolerus* panzer. In this present communication two species are being added new to science. A revised key to all 9 species from India is provided.

Type material of new species is housed at Division of Entomology, Pusa National collection, Indian Agricultural Research Institute, New Delhi, India.

Abbreviations

AWMT - Apical Width of Metatibia, EL - Eye Length, IATS - Inner Apical Tibial Spur, ICD - Inter Cenchri Distance, IDMO - Interocular Distance at Level of Median Ocellus, ITD - Inter Tegular Distance, LID - Lower Interocular Distance, OATS - Outer Apical Tibial Spur, OCL - Ocellooccipital Line, POL - Postocellar Line, UCL - Oculooccipital line, UOL - Oculoocellar Line.

Key to Indian species of *Dolerus* panzer

1. Clypeus triangularly incised upto $\frac{1}{2}$ of its medial length 2

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Clypeus triangularly very shallowly incised (i.e. upto $\frac{1}{4}$ of its medial length) ... *D. infuscata* (Saini & Singh, 1987)

2. Tegula black 3
   Tegula yellow 4
3. Abdomen entirely black ..... *D. darjeelingi* (Saini & Singh, 1987)
   Extreme apical margins of tergites 2–7 whitish; posterior margins of tergites 8 and 9 yellowish brown ..... *D. mira* (Muche, 1983)
4. Abdomen entirely black ..... *D. manalii* (Saini & Singh, 1987)
   Abdomen not entirely black 5
5. Mesonotum entirely black 6
   Mesonotum not entirely black 7
6. Abdominal segments 3 and 4 auratus ..... *D. cingulatus* sp. nov.
   Abdominal segments 2–6 ferruginous .... *D. ferruginosa* (Saini & Singh, 1987)
7. Mesopleuron entirely black 8
   Mesopleuron not entirely black ..... *D. tangmargensis* (Saini & Singh, 1987)
8. All abdominal tergites (except 3 apical tergites in male only) and all sternites ferruginous ..... *D. nigripleuris* sp. nov.
   Abdominal tergites 2–6 and respective sternites rufous .....
   D. rufocinctus Cameron, 1876

***Dolerus cingulatus* sp. nov**

(Figs. 6–8)

Female: Colour: Body black, auratus are: tegula, a band on abdominal segments 3 and 4, a spot on tergite 9, extreme apices of all femora, basal $\frac{2}{3}$ of all tibiae. Wings uniformly infumated, venation including costa, subcosta and stigma black.

Structure

Average length 7 mm. Antenna long, subincrassate in middle, 1.8x head width; scape longer than broad, pedicel as long as its apical width, segment 3 longer than 4 as 3:2, clypeus triangularly incised upto $\frac{1}{2}$ of its medial length (Fig. 7), labrum broader than long as 3:2, with roundly pointed anterior margin, malar space 0.5x diameter of median ocellus, lower margins of eyes below level of antennal socket, LID:IDMO:EL = 3:3:2, postgenal carina present, hind orbits faintly carinate, frontal area slightly above level of eyes, supraantennal tubercles and frontal ridges insignificant, median fovea absent; post-, inter- and circumocellar furrows inconspicuous; lateral furrows quite distinct, deep, parallel and abruptly ending just before hypothetical hind margin of head; postocellar area subconvex, broader than long as 3:2, head narrowing behind eyes, POL:OCL:UOL:UCL = 1:1:1:1, mesoscutellum subconvex, appendage carinate, ICD:ITD = 1:4, tarsal claw (Fig. 6) with a dent-like subapical tooth and without basal lobe, metabasitarsus equal to following 3 joints combined, IATS:AWMT:OATS = 5:4:4. Lancet (Fig. 8) having 10 serrulae.

Sculpture

Head covered with large, deep and dense punctures; punctures on mesonotum similar to those on head but mesopleuron rugose; mesosternum having smaller punctures with shining surface between them; scultellum punctured like head; stripes along pleural sutures impunctate, postscutellum though punctured, the punctuation not as dense as of scutellum. Abdomen shining and impunctate.

Pubescene

Body covered with silvery pubscence.

Male

Yet to discover.

Material examined

Holotype: Female, Nagaland, Satakha, 1500 m, 15.v.1993, Coll. V. Vasu. Paratype: Female, Nagaland, Pfutsero, 2000 m, 18.v.1993, Coll. V. Vasu.

Distribution

India: Nagaland

Diagnosis

On account of broad key characters *D. cingulatus* allies with *D. ferruginosa* but remains far apart from the latter as well as from other species discussed under this genus on account of some significant characters such as, abdominal segments 3 and 4 auratus only; tegula yellow; antennal segments 3 and 4 as 3:2; malar space $\frac{1}{2}$ the diameter of median ocellus, and lateral furrows parallel.

Etymology

Species name pertains to coloured band on abdominal segments 3 and 4.

Dolerus nigripleuris sp. nov.

(Figs. 1–5)

Female: Colour: Body auratus, black are: antenna, head, posteroventral margin of pronotum, medial suture of mesonotal middle lobe; mesoscutellum, appendage; metascutellum, mesosternum, meso- and metapleura, pro- and mesocoxae more or less, basal $\frac{1}{2}$ of metacoxa, all trochanters and adjacent parts of femora more or less, apex of metatibia, all tarsi more or less. Wings infumated; vanation including costa, subcosta and stigma piceous.

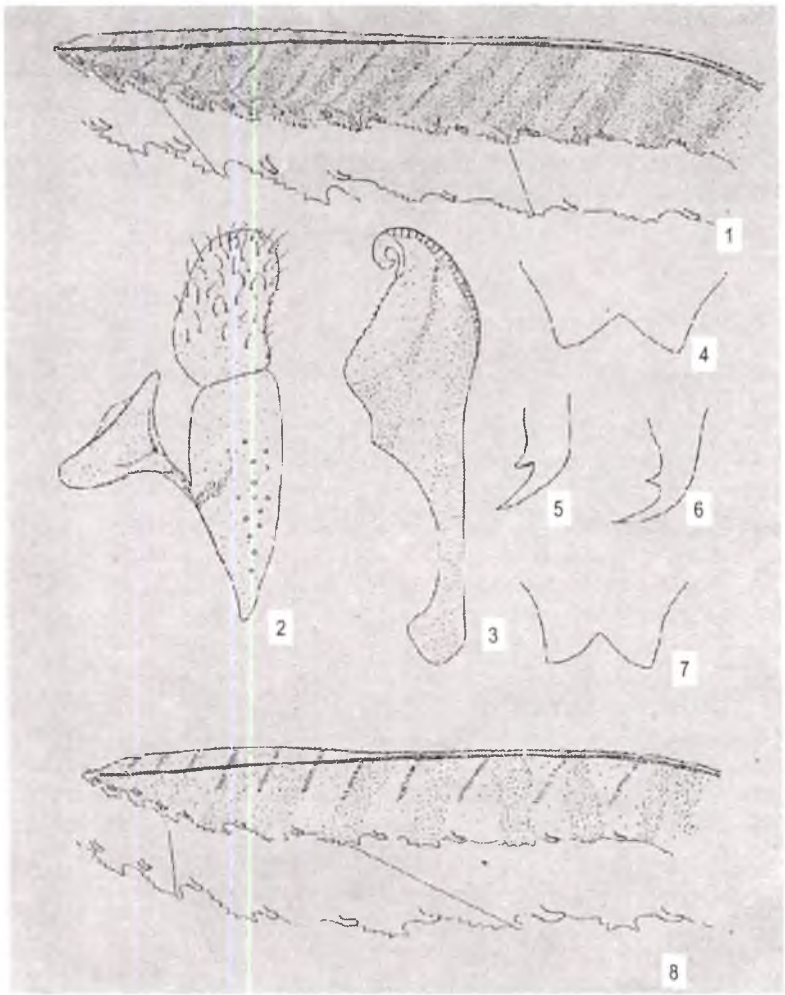

Fig. 1. Lancet of *Dolerus nigripleuris;* 2. Gonoforceps of *D. nigripleuris;* 3. Penis valve of *D. nigripleuris;* 4. Clypeus of *D. nigripleuris;* 5. Tarsal claw of *D. nigripleuris;* 6. Tarsal calw of *D. cingulatus;* 7. Clypeus of *D. cingulatus;* 8. Lancet of *D. cingulatus.*

Structure

Length 7.5 mm. Antenna subincrassate in middle, 1.7x head width, scape longer than broad, pedicel as long as its apical width, segment 3 longer than 4 as 3:2, clypeus (Fig. 4) roundly incised upto $\frac{1}{2}$ of its medial length, labrum broader than long as 2:1, with rounded anterior margin, malar space 0.5x diameter of median ocellus, lower margin of eyes below level of antennal socket, LID:IDMO:EL = 3:3:2, postgenal carina present, hind orbit ecarinate, frontal area almost at level of eyes, supraantennal tubercles and frontal ridges insignificant; median fovea in form of shallow pit just above antennal bases; post-, inter- and circumocellar furrows indistinct; lateral furrows indicated like streaks, parallel and ending well before hypothetical hind margin

of head; postocellar area subconvex, broader than long as 3:2, head narrowing behind eyes, POL:OCL:UOL:UCL = 4:3:4:4, mesoscutellum subconvex, appendage ecarinate, ICD:ITD = 1:4; tarsal claw (Fig. 5) with a subapical tooth distinctly shorter than apical one, basal lobe absent, metabasitarsus equal to following 3 joints combined, IATS:AWMT:OATS = 6:4:5. Lancet (Fig. 1) having 13 serrulae.

Sculpture

Head with large, dense, prominent punctures, intervening spaces shining, mesonotum with dense, minute, irregular punctures, surface shining, mesoscutellum punctured like head, mesepisternum rugose with large, dense, pit–like confluenting punctures; mesosternum punctured like mesonotum, surface shining.

Pubescence

Body covered with golden pubescence except blackish parts where it appears to be silvery.

Male

Length 6.5 mm. Similar to female except: ventral $\frac{1}{2}$ of pronotum and apical 3 abdominal segments black; apical $\frac{1}{2}$ of procoxa and all trochanters more or less auratus. Genitalia: Penis valve (Fig. 3), gonoforceps (Fig. 2).

Material examined

Holotype: Female, Jammu & Kashmir, Tangmarg, 2000 m, 20.vi.1987, Coll. M. S. Saini. Paratype: 1 male with same data as holotype.

Distribution

India: Jammu & Kashmir

Diagnosis

Entirely black mesopleuron, yellow tegula, deeply triangularly incised clypeus, POL:OCL:UOL:UCL = 4:3:4:4, ratio of antennal segments 3 and 4 as 3:2 and unique colour pattern of abdomen make *D. nigripleuris* so distinct from rest of the species of this genus that it deserves the status of a new species.

Etymology

Species name refers to blackish mesopleuron.

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Short Communication

Foraging Strategies in the Ants *Myrmicaria brunnea* and *Diacamma ceylonense*-Some Preliminary Observations

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Abstract: *Myrmicaria brunnea* forager communicates by means of chemical and/or acoustic signals so that other foragers present nearby can move towards it and find the bait sooner than they would on their own. However, this sort of communication seem to have not been present in *Diacamma* sp. foragers

The social organization of recruitment and food retrieving in ants and other social insects is an important component in the study of their ecology and sociobiology. In our preliminary survey of the ants of the campus of the Indian Institute of Science, Bangalore (Rastogi *et al.* 1997), we came across the following interesting phenomena.

In a series of experiments, we provided 10 *Corcyra cephalonica* (Saint.) (Lepidoptera: Pyralidae) larvae in a clump at one meter distance from the nests of *Diacamma ceylonense* and *Myrmicaria brunnea*. In the case of *D. ceylonense*, the bait was discovered by a forager within 15.7 ± 17.6 (n = 13) minutes and in the case of *Myrmicaria* it was discovered within 15.6 ± 11.2 (n = 8) minutes. In both cases, although, much more predictably in *Myrmicaria*, additional foragers moving around the bait also subsequently discovered the bait (Table 1 and 2).

In the experiment with *Myrmicaria brunnea* it was our impression that, once a first forager discovered the bait, other foragers arrived in relatively quick succession. If our impression is correct, it suggests that the forager arriving first, somehow communicates with other foragers searching nearby and makes it possible for them to reach the bait rapidly. To test if our impression was correct, we conducted the following analysis. If there was no communication between the forager which arrived first and those that arrived later, then the time taken by any forager to discover the bait should come from the distribution of the times taken by the first foragers to discover the bait. Using the distribution of times taken by the first foragers to discover the bait, we simulated

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Table 1
Time taken by each *Diacamma ceylonense* foragers to reach the bait (in minutes). For all foragers, time to reach the bait is calculated from the time the bait was provided.

Expt.	Foragers					
No.	1st	2nd	3rd	4th	5th	6th
1	12	100				
2	69	98				
3	9	12	40	50	89	100
4	20	60	78	91		
5	12	62	78			
6	2	28				
7	4	28				
8	6	32				
9	28	59				
10	6	10				
11	4	22	42	50	68	74
12	16	60	80			
13	16	28				

Table 2
Time taken by *Myrmicaria brunnea* foragers to reach the bait (in minutes). For all foragers, time to reach the bait is calculated from the time the bait was provided.

Expt.	Foragers				
No.	1st	2nd	3rd	4th	5th
1	35	36	36	37	37
2	26	27	27	27	27
3	12	12.5	13	13	13
4	21	21.5	22	23	23
5	2	3	3	3	3
6	15	17	17	17	17
7	10	12	12	12	12
8	4	5	5	5	5

times required by 40 foragers (5 foragers at each of 8 baits as observed in the experiment) and computed the time intervals between the arrival of successive foragers at each bait. These time intervals between the arrival of successive foragers in the simulated data were compared with time intervals between the arrival of successive foragers in the observed data by at t-test. The simulation was repeated 1000 times and in all cases the time intervals between the arrival of successive foragers in the simulation were significantly higher than corresponding the values in the observed data (equivalent to $p < 0.001$). We suggest therefore that the first *Myrmicaria brunnea* forager

Table 3
Time intervals in the arrival of successive foragers

		Sample size	Min.	Max.	Mean	S.D.
Myrmicaria	Observed	32	0	2	0.37	0.58
brunnea	Simulated	32000	0	48.49	6.88[1]	6.17
Diacamma	Observed	25	3	88	23.76	17.76
ceylonense	Simulated	25000	0	96.85	14.48[2]	19.80

[1] In the case of *Myrmicaria brunnea*, in all 1000 simulations, the time interval between the arrival of successive foragers was significantly greater (t–test, $p < 0.05$) than in the observed data. Thus the null hypothesis that the time interval in the observed data is equal to that in the simulated data is rejected at $p < 0.001$.
[2] In the case of *Diacamma ceylonense*, in 131 out of 1000 simulations the time interval in the arrival of successive foragers was not significantly greater (t–test, $p < 0.05$) than in the observed data. Thus the null hypothesis that the time gap in the observed data is equal to that in the simulated data is accepted at $p > 0.13$.

communicates, perhaps by means of chemical and/or acoustic signals, so that other foragers present nearby can move towards it and find the bait sooner than they would on their own. When we conducted a similar simulation and analysis of the data on the rates at which *Diacamma ceylonense* foragers discovered baits, we found that in 131 out 1000 simulations (equivalent to $p > 0.13$) there was no difference between the observed and simulated time intervals in the arrival of successive foragers. Thus our data do not suggest that *Diacamma ceylonense* foragers communicate in a similar fashion. Descriptive statistics of observed and simulated time intervals between the arrival of successive foragers for *Myrmicaria brunnea* and *D. ceylonense* are given in Table 3. Remarkable as it is, the possible short-range communication performed by *Myrmicaria brunnea* is not surprising because some species of ants are known to use chemical and acoustic signals for similar short-range communication (Markl and Hölldobler 1978; Hölldobler *et al.* 1978; Hölldobler and Wilson, 1990).

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Short Communication

Thermal Acclimation in the Endogenous Respiratory Rate of Fatbody of Eri-silkworm *Philosamia ricini.*

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Abstract: The fat body of eri-silkworm demonstrates an excellent capacity of thermal acclimation in the rate of its endogenous oxygen consumption. The endogenous respiratory rate of fat body of cold adapted silkworm is always higher than those of warm adapted insects, at all the temperatures tested in this study. This pattern of thermal acclimation may be categorized as almost "translational" (Prosser pattern II A) with a slight tendency of "rotation" towards higher temperature (Prosser pattern IV-A) indicating higher involvement of a "quantitative" change with little implication of a "qualitative" one in the enzyme system of tissue.

Keywords: Fatbody tissue, eri-silkworm, endogenous oxygen consumption.

Temperature determines life of organism more than many other environmental factors. The cause of this temperature sensitivity lies in the fundamental facts that all organisms are built up by chemical reactions which follows the laws of thermodynamics (Alexandrove, 1977).

A perusal of literature reveals that a number of insects species exhibit either 'complete' or 'partial' pattern of respiratory compensation against thermal fluctuation at organismal level (Singh and Das, 1977, 1980; Hunter, 1964, '65, '66 and '68). Meyer and Schavb (1973) have confirmed these results with experiment on oxygen consumption of larval stage of Calliphoridae. However, counter efforts have been made regarding exploring the capacity of thermal acclimation of oxygen consumption at the cellular level of insects (Thiessen and Mutchmore, 1967; Das and Singh, 1974; Singh *et al.* 1988; Singh and Singh, 1992 and Singh 1994). Accordingly, the present communication reports the thermal acclimatory responses of fat body tissue of eri-silkworm *Philosamia ricini* of both cold and warm adapted.

The eri-silkworm were reared in the laboratory at 23±1° C (Singh and Singh, 1984). The laboratory reared worms were transfered right on the first day of 3rd instar in BOD incubator maintained at 15 ° C and at 30° C with relative humidity of 75%. Thus the period of 3rd and 4th instar larval duration was available to the insects for

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Table 1
Effect of temperature on endogenous respiratory rate (μl O_2 consumed/hour/mg protein) of fat body of cold and warm adapted eri silkworm.

Temperature of measure-	Nature of adaptation	DEVELOPMENTAL STAGES					
		INSTAR V SPINNING PERIOD					
		Early	Middle	Late	Early	Middle	Late
	Cold adapted	0.279 ±0.013	0.348 ±0.011	0.740 ±0.030	0.583 ±0.020	0.250 ±0.009	0.129 ±0.017
15° C	Warm adapted	0.167 ±0.012	0.213 ±0.013	0.474 ±0.014	0.360 ±0.009	0.158 ±0.013	0.078 ±0.008
	Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
	Cold adapted	0.523 ±0.018	0.654 ±0.210	1.344 ±0.039	1.030 ±0.087	0.450 ±0.030	0.231 ±0.011
25° C	Warm adapted	0.365 ±0.016	0.465 ±0.018	1.010 ±0.053	0.750 ±0.024	0.323 ±0.014	0.160 ±0.010
	Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
	Cold adapted	0.862 ±0.038	1.050 ±0.083	2.386 ±0.067	1.667 ±0.083	0.743 ±0.035	0.403 ±0.013
35° C	Warm adapted	0.662 ±0.021	0.888 ±0.031	1.859 ±0.110	1.408 ±0.118	0.563 ±0.034	0.305 ±0.016
	Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

thermal adaptation. The fat body tissue were dissected out according to Singh and Singh, (1992). The endogenous respiratory rate of fat body was measure according to methods described by Umbreitt *et al.* (1964). The measurements of the rate of oxygen consumption of the tissue of cold and warm adapted insects were made at 15° C, 25° C and 35° C. The rate of endogenous oxygen consumption of fat body was expressed as μl oxygen consumed/hour/mg protein.

The thermal coefficient (Q10) for the endogenous respiratory rate in a particular thermal range was calculated as Van,t Hoff's equation. Experiment was repeated three times with nine replications. The significance of all the data was statistically tested by standard "t" test.

Table 1. demonstrates the effect of temperature on the endogenous respiratory rate of fat body of cold and warm adapted insects measured at three different temperature (15° C, 25° C and 35° C) during the 5th instar and spinning period . The fat body of cold adapted insects exhibits higher values of respiratory rate than those of warm adapted at all temperature of measurements.

The degree of difference in the respiratory rate between the two thermal groups of the insects of each stage is observed to decrease gradually with an increase in ambient temperature as it evident from Table 2. However percent increase in the endogenous respiratory rate of fat body due to cold adaptation remains almost static irrespective of developmental stage, when measured at 15° C and 25° C, but in contrast at 35° C, it is

Table 2
Per cent increase in the endogenous respiratory rate of fat body of eri silkworm due to cold adaptation measured at different temperature (Computed from the data in Table 1)

Temperature of measurement (° C)	DEVELOPMENTAL STAGES					
	INSTAR V SPINNING PERIOD					
	Early	Middle	Late	Early	Middle	Late
15° C	67.1	63.4	56.1	61.9	58.2	65.4
25° C	43.3	40.6	33.1	37.3	39.3	44.3
35° C	30.2	18.2	28.5	18.4	32.0	32.1

variable at different developmental stages (Table 2.). Table 3 illustrates the marginal change in the value of Q10 for the endogenous respiratory rate of fat body due to adaptation of insects to low and high temperatures. A tendency of slight decrease in Q10 value is observed due to cold adaptation in the lower and higher thermal range for the insects of all developmental stages.

Almost a similar observation can be noticed when the comparison is made between the Q10 values of lower and higher thermal range irrespective of thermal acclimation in each developmental stage. Oxygen consumption is often taken as a measure of overall metabolic rate of an animal. Its measurements has been employed more than any other experimental parameters to monitor changes in insect metabolism associated with temperature (Keister and Buck, 1974). In the present study the endogenous respiratory rate is always higher in cold adapted worms in all developmental stages and at all temperatures. This type of thermal response can be categorized as 'translational' pattern of metabolic compensations (Prosser Pattern II-A), but partial (Precht type III). Similar observations were made by Meyer and Schavb (1973), in larval stage of Calliphoridae. However, a marginal tendency towards rotation with intersections with rate versus temperature (R-T curve) at a high temperature (Prosser pattern IV A) may be noticed. This is further supported by the slight reduction in value of Q10 during cold adaptation (Table 3). The extent of increase in the endogenous respiratory rate of the fat body tissue due to cold adaptation presents a gradual decrease with an increase in the ambient temperature (Table 2).

It is evident that metabolic strategy employed by the fat body of the insects for thermal acclimation is independent of the stage of its development. Moreover, at each temperature of measurement viz; 15, 25 and 35° C the degree of difference in the endogenous respiratory rate of fat body between the two thermal categories remains more or less of same magnitude irrespective of developmental stage (Table 3).

The degree of compensation of the metabolic rate at higher temperature of measurement (35° C) is lesser than what is seen at lower temperature (15 and 25° C). Obviously insect possess a better capacity of cold adaptation than warm adaptation. Perhaps, a high temperature like 35° C is not suitable for these insects for their normal growth and development (Sachan and Bajpai, 1973). While a 'translational' pattern of thermal acclimation involves a quantitative strategy, the 'rotational' pattern signifies more of a qualitative, one during thermal acclimation of poikilotherms (Prosser, 1973). Since, the pattern of thermal acclimation exhibited by the tissue at each devel-

Table 3
Approximate Q10 values for the endogenous respiratory rate of fat body of cold and warm adapted eri silkworm.

Periods	Nature of adaptation	DEVELOPMENTAL STAGES			
		INSTAR V		SPINNING PERIOD	
		Thermal Range		Thermal Range	
		15-25° C	25-35° C	15-25° C	25-35° C
Early	Cold adapted	1.88	1.65	1.77	1.62
	Warm adapted	2.18	1.81	2.08	1.62
Middle	Cold adapted	1.88	1.60	1.80	1.62
	Warm adapted	2.18	1.91	2.04	1.62
Late	Cold adapted	1.82	1.78	1.79	1.62
	Warm adapted	2.13	1.84	2.05	1.62

opmental stage is observed to be 'translational' with a slight tendency of rotation, it appears that the metabolic strategy employed by the fat body tissue involves more of quantitative change than qualitative. Probably, this may be the reason why a marginal decrease in Q10 value supports the contention of Rao and Bullock (1954) regarding an adaptive significance of lowered Q10 of biological rate process during cold acclimation, which results in an increased thermal insensitivity. Besides, a critical analysis of data (Table 3) demonstrates a diminution in the value of Q10 for the endogenous respiratory rate of fat body with increasing temperature of measurements for any of those thermal categories of a particular developmental stage of the insect. This agrees with the study of Bullock (1955).

Thus it may be concluded from the aforesaid observations that the fat body tissue of eri-silkworm possess an excellent capacity of thermal acclimation in its metabolic rate.

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ANNOUNCEMENT

XVI NATIONAL SYMPOSIUM ON REPRODUCTIVE BIOLOGY AND COMPARATIVE ENDOCRINOLOGY 1998

The XVI National Symposium of SRBCE will be held in Thiruvananthapuram during January 1998

Abstracts from Basic, Clinical and Comparative Endocrinologists in the field of Reproduction, Contraception, Hormone Biotechnology, Hormone Physiology, Hormone Chemistry, Immunoendocrinology and Environmental Endocrinology are invited.

The Scientific Programme will consist of Professor Pera Naidu Govindarajulu Gold Medal Oration, State of Art Lectures, Symposium Lectures and peer reviewed Oral and Poster presentations.

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